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Vol. 32 **November, 1955** **No. 6**

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The next chapter titled: "European Millwrights" will be published soon.

*This is the title of a definitive history of milling by John Storch and Walter Dorwin Teague, published by the University of Minnesota Press at Minneapolis and copyrighted by the University of Minnesota. It is used with permission as a source of material for this series of advertisements.

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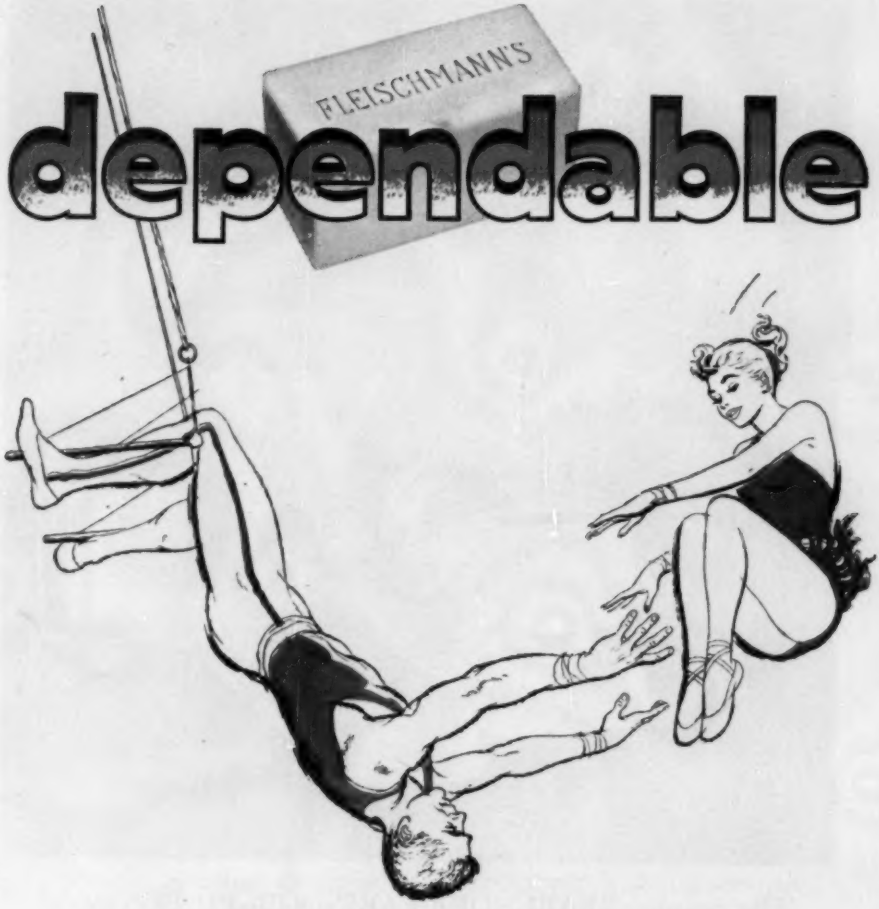

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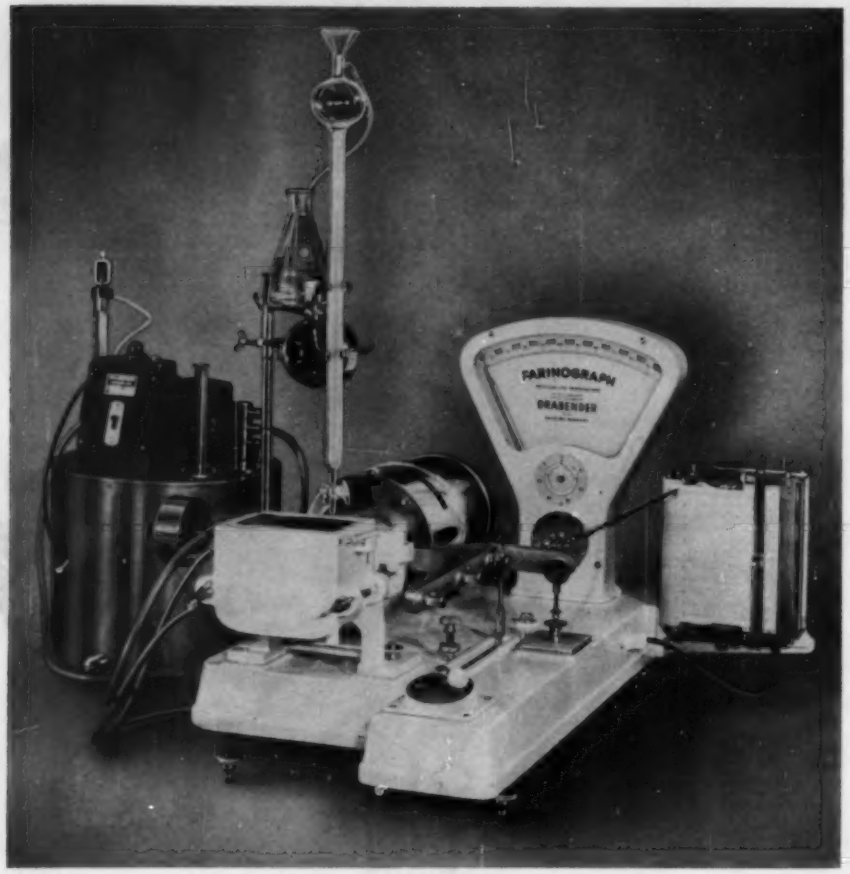

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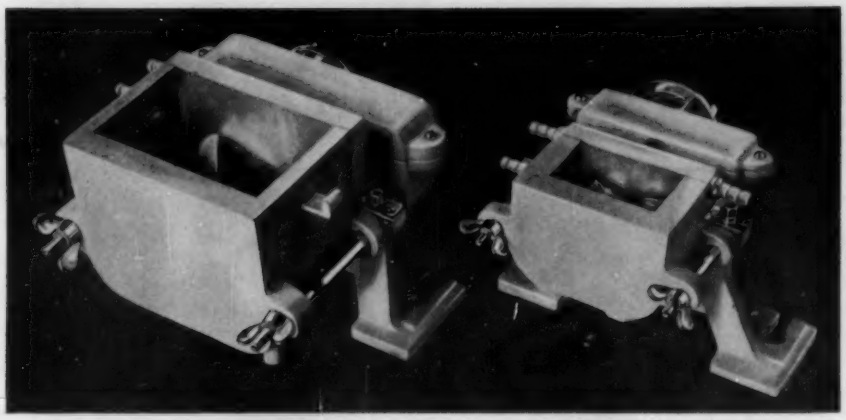

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CEREAL CHEMISTRY

VOL. 32 NOVEMBER, 1955 NO. 6

SOME BIOCHEMICAL FACTORS INVOLVED IN THE RESISTANCE OF THE WHEAT PLANT TO ATTACK BY THE HESSIAN FLY[1]

FAWZY Y. REFAI,[2] ELMER T. JONES,[3] AND BYRON S. MILLER[4]

ABSTRACT

Neither respiration activity of the wheat plant stem nor hydrogen-ion concentration of plant cell sap from different wheat varieties was correlated with degree of resistance to attack by hessian fly. Differences in protein, ash, cellulose, silica, and other trace mineral contents of plants of different wheat varieties also were not correlated with resistance.

Hemicellulose content of the wheat plant and degree of resistance were positively correlated. Stems of resistant varieties also were more resistant to shear than stems of susceptible varieties.

Larvae were demonstrated *in vitro* to secrete hemicellulase as well as something that inactivated or inhibited wheat plant phosphorylase. This was substantiated by an accumulation of sugars in leaves of infested plants.

The hessian fly, *Phytophaga destructor* (Say), was first reported to damage wheat in Kansas in 1871, ninety-two years after its first serious ravages on Long Island, N. Y. From this date, fly damage increased and was widespread and generally recognized by 1883–1884. The annual loss during the period 1942–1951 is estimated at 9,389,000 bu. of wheat valued at about $16,826,000 (25). Damage estimated at $100,000,000 in a single year has been caused by this pest (5).

During recent years no serious general outbreaks of hessian fly have occurred because the prevailing low humidity and high temperature conditions have been unfavorable for emergence, infestation, and development of the insect. These unfavorable environmental conditions, together with the extensive acreage of semiresistant Pawnee wheat grown during the last ten years in Kansas, have limited the loss due to hessian fly infestation.

[1] Manuscript received May 6, 1955. Cooperative investigations of the Field Crops Research Branch and the Entomology Research Branch, Agricultural Research Service, U. S. Department of Agriculture, and the Department of Flour and Feed Milling Industries, Kansas Agricultural Experiment Station, Manhattan. Contribution No. 253, Department of Flour and Feed Milling Industries, Kansas Agricultural Experiment Station, Manhattan. A portion of the work presented here was included in a dissertation submitted by the senior author to the Graduate School, Kansas State College, in partial fulfillment of the requirements for the degree, Doctor of Philosophy, May, 1955.

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A maze of genetic, anatomic, physiologic, and ecologic evidences and theories has been offered to explain the factors involved in resistance or susceptibility of wheat varieties to attack by the hessian fly. Unfortunately, however, few data are available that have a bearing on the problem.

Painter (17) found that the hessian fly population of any one locality consisted of a mixture of two or more strains, which differed in their ability to infest different varieties of wheat. Painter *et al.* (19) observed that soft wheats provide the main genetic sources of resistance to the hessian fly. The reason for this may be that soft wheats have been subjected to natural and artificial selection, including variety testing and plant breeding, for almost 100 years longer than have the hard winter wheats.

McColloch and Salmon (15) indicated that resistance resulted from physiological conditions present at the base of the plant. This was based on their observation that larvae developed high in the stem of resistant plants and not at the base of the plants. Undeveloped larvae generally were found at the base where the leaf sheath has its origin. Later, Jones (14) obtained evidence that resistance or susceptibility to the hessian fly could be influenced by the nature and growth of the plant tissue. Larvae appeared to be killed by the greater pressure exerted on the tissues of the resistant varieties. On susceptible plants larvae proceeded to develop rapidly, whereas on resistant ones they usually were destroyed in the red larval stage. In most cases, when the red larvae succeeded in passing the first molt on resistant plants, they often developed high up on the stems at the front of the leaf sheaths or in similar locations of least pressure of the growing stem against the leaf sheath.

Fly-resistant grasses and wheat plants generally, though not always, appear to have coarser and more closely spaced vascular bundles, and heavier, more rigid cellular structure than susceptible plants (14). A possible relationship between cellular structure and hessian fly infestation is indicated further by the fact that some cereals such as rye and barley (13) are highly resistant or semiresistant to attack by the fly. Rye, which is highly resistant, contains relatively large amounts of alpha-cellulose (21). Oats are not attacked by hessian fly.

The silica content of the wheat stem is believed by some to be related to the stiffness of the wheat stem and perhaps to resistance to attack by the hessian fly. Enock (9) stated that wheat stems containing a large amount of silica were not injured severely by larvae. McColloch and Salmon (15) found that several very susceptible varieties showed marked resistance when grown in Pfeffer's solution containing

a small amount of sodium silicate. The degree of resistance increased with the amount of silicate added. The amount of silica has been considered by Painter (18) to be related to resistance through strengthening of the cell walls. Painter suggested that it might also act by adsorbing digestive enzymes from the insect.

Parker and Painter (20) pointed out that a protoplasmic factor appeared to be involved in the resistance of certain wheats. Recently, Painter (18) has suggested that an enzymatic system may be involved, since the larvae may secrete enzymatic or toxic substances which, in the case of susceptible varieties, penetrate several layers of plant cells and arrest cell growth immediately beneath the developing larvae.

A partial solution to the problem of hessian fly destruction has been achieved by breeding resistant varieties of wheat (5). The wheats of the world have been screened for desirable resistant parental material, which includes Durum (P. I. 94587), Illinois No. 1W38, Marquillo, and Kawvale wheats. These varieties have been crossed with varieties adapted to a given area and tested for two to three generations to isolate a few desirable plants which are then crossed, tested, and recrossed with plants having characteristics which are lacking. In this way a satisfactory resistant variety is developed. Each generation must be tested to eliminate undesirable material.

Elimination tests are made either in field nurseries or in greenhouses. Short rows of test strains are planted and infested with hessian flies. Upon maturity of the insects, the plants are critically examined for infestation by experienced entomologists. Promising varieties receive critical examination for both fall and spring reaction to the pests. Seed from sister lines from the same plant are tested simultaneously in several nurseries, because resistance is affected by environmental conditions and some varieties are resistant only to certain strains of flies. On the basis of records taken at several nurseries, desirable varieties are finally tested in a master nursery. The hard red winter wheat varieties, Pawnee and Ponca, and several unreleased varieties are notable achievements in this work.

In contrast with progress made in breeding wheats resistant to hessian fly, little or no progress has been made toward studying the biochemical factors correlated with degree of resistance to attack by the hessian fly. Furthermore, no satisfactory explanation of the phenomena of resistance has been advanced in the voluminous literature dealing with the hessian fly.

Knowledge concerning the biochemistry of the wheat plant would be valuable in determining the resistance of new wheat varieties and might eventually be of use in devising means of controlling the fly

population. The present study was designed to explore specific areas for further, more intensive investigations on the biochemical basis of resistance in wheat to infestation by the hessian fly.

Materials and Methods

Wheat Varieties Used. The resistant winter wheats used in this study are the result of 20 years of testing and elimination of undesirable plants. They include Pawnee × 94587 (C. I. 12855), Mediterranean-Hope-Pawnee × Oro-Illinois No. 1W38-Comanche (C. I. 12804 and C. I. 12872), Ponca (Kawvale-Marquillo × Kawvale-Tenmarq), Marquillo-Oro × Oro-Tenmarq (C. I. 12854), and Pawnee (Kawvale × Tenmarq). In addition, the following susceptible varieties were used as a basis for comparison: Comanche (Oro × Tenmarq), Tenmarq, Oro, Mediterranean, Triumph, Epidor, Hope, Ceres, and Rival.

Whenever possible from the standpoint of availability of both planting space and seed, a series of different crosses and their parent varieties was used. Otherwise only selected resistant and susceptible varieties were planted and tested. In most cases analyses were performed only on the 1.5-in. portion of the stem just above the root system. This is the part of the plant where the larvae feed. It was believed that analysis of this section would provide the greatest opportunity for studying the biochemical factors that determine resistance.

The varieties used in this work can be arranged according to field observations into five groups of descending order of resistance (Table I). Each group is composed of varieties demonstrating similar reactions toward attack by the fly.

TABLE I

WHEAT VARIETIES DIFFERENTIATED BY FIELD PERFORMANCE ACCORDING TO THEIR DEGREE OF RESISTANCE

DEGREE OF RESISTANCE	VARIETY	
	Winter Wheats	Spring Wheats
1	CI 12855	PI 94587 (Durum)
2	CI 12872, CI 12804	Illinois No. 1
3	Ponca, CI 12854	Marquillo
4	Pawnee, Kawvale	
5	Comanche, Tenmarq, Oro, Mediterranean, Triumph, Epidor	Hope, Ceres, Rival

The highest order of resistance is indicated by the number 1, and progressively lower orders of resistance, by the numbers 2, 3, and 4. Pawnee and Kawvale represent the lowest order of resistance. Suscepti-

ble varieties are indicated by the letter S.

Source of Hessian Fly. Active larvae were obtained from cultures maintained by one of the authors at the Entomology Research Branch hessian fly laboratory, Manhattan, Kansas. The Marshall County Kansas biological strain of fly was used.

Method for Testing the Rigidity of the Wheat Plant Stem. A simple cutting device was designed to test the rigidity of wheat plant stems from different varieties. The details of the instrument are shown in Fig. 1. The instrument consisted of a stationary board *A*, and a moving part *B* made up of two boards and three metal sheets of the same length and height as parts *A* and *B*. A notch in the center metal sheet held a single-edged razor blade. The pan, which was attached to part *B* by a string running over a pulley, was equipped with a paper cup and was padded with a piece of sponge rubber to absorb the effect of fall-

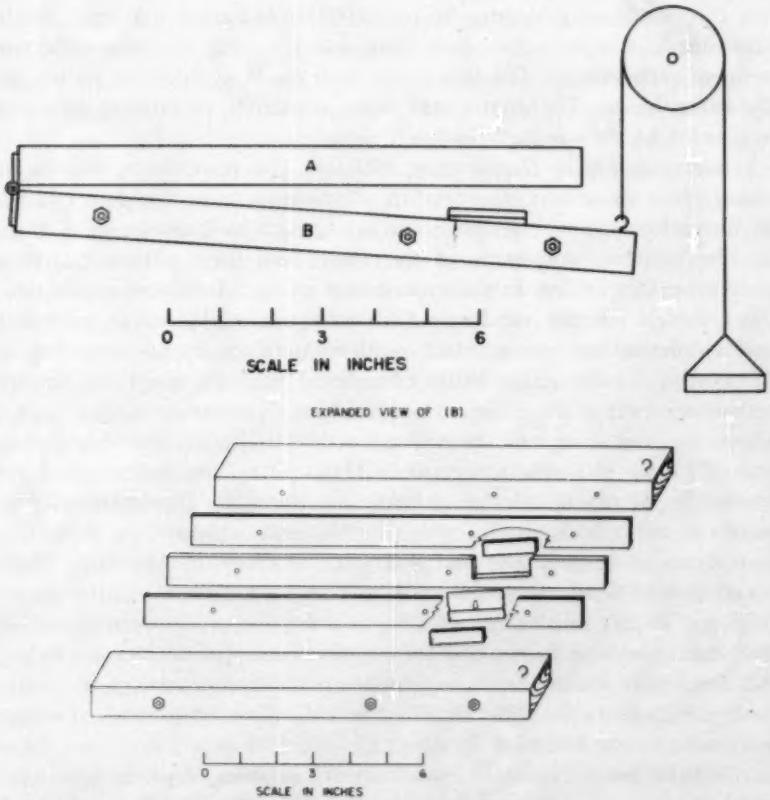

Fig. 1. A simple cutting device designed to test the resistance of wheat plant stems to shear.

ing lead shots. A small projection on the underside of part *A* caused the cut to be made at a fixed distance above the crown of the plant.

The following procedure was used in all measurements designed to measure the rigidity of wheat plant stems. The flats in which the plants were grown were brought into the laboratory the same day rigidity measurements were to be performed. Plants were removed from the soil immediately before testing. The outside sheath was removed, and a 1-in. section of the stem above the root system was cut off with a razor blade. The diameter of the stem was measured with a micrometer. Only those stems having uniform diameters within ± 0.005 in. were selected. This part of the stem was placed at the bottom of board *A* so the blade would bisect the stem. Lead shots were added gradually until the stem was cut. The cutting of the stem was indicated by a short sudden movement of the cutting edge of the razor blade. The weight of the lead shot used was recorded, and resistance to shearing was calculated as g. of weight per 0.001 in. of stem diameter. Each razor blade was used for seven cuts, one for each of seven different stems of each variety. The seven cuts were made at different points on the razor blade. To obtain maximum similarity of cutting effect, a new razor blade was used for each sample.

Determination of Respiration Activity. The respiration activity in wheat plant tissue was measured in 13 varieties using the direct method for carbon dioxide determination described by Umbreit *et al.* (24).

The varieties, consisting of six crosses and their parent varieties, were grown in 13 flats in the greenhouse using a balanced incomplete block design (6). Six randomized varieties were planted in each flat and a different flat was planted on alternate days. Plants were dug at the end of 15 days, this being considered the best stage for testing resistance of the plants to insect attack. A small section of stem (20 mg.) above the root system of the plant, but not including the sheath, was soaked for 15–18 hours in Sorensen's M/60 phosphate buffer (pH 5.91) containing 60 mg. of calcium chloride per liter (10). Duplicate samples were taken for both oxygen consumption and carbon dioxide evaluation determinations. The samples were inserted in Warburg flasks together with 5 ml. of Sorensen's buffer. A folded piece of filter paper (0.75 × 0.75 in.) and 0.4 ml. of 20% sodium hydroxide were placed in the center wells of flasks used for oxygen consumption measurements. All flasks were flushed with oxygen prior to being placed in the water bath which was controlled at 35° ± 0.01°C. The manometer readings were taken each hour for 5 hours.

Preparation of Holocellulose (2, 3). All samples of wheat stem were ground to pass a 25-mesh screen but were retained by a 50-mesh

screen. A 3-g. portion was extracted first in a Soxhlet apparatus with alcohol-benzene (1:2) and then with 0.5% ammonium citrate at 80°C. The residue was added to water treated with sodium chlorite and acetic acid to remove the lignin, heated, filtered, and washed with water. The resulting nearly completely white holocellulose was dried in an air oven at 105°C. for 1 hour.

Estimation of Hemicellulose (3). Dried holocellulose (0.2 g.) was extracted with aqueous alkali and the resulting solution oxidized completely with acid chromate solution. The reduced chromate served as an index of the organic matter present and was determined spectrophotometrically using light of 600 mμ wave length. A standard curve was established which related the reduced chromate to the quantity of glucose present.

Determination of Hemicellulase Activity.[5] The buffer employed was a solution made up of 10 ml. of 0.1*N* citric acid, 8 ml. of 0.2*M* dibasic potassium phosphate, and 82 ml. of distilled water (pH 4.75). A 10-ml. aliquot of a 1% suspension of hemicellulose in buffer was placed in a glass-stoppered 125-ml. Erlenmeyer flask and 10 ml. of buffer in a second flask to serve as a blank. The flasks were stoppered and placed in a water bath at 45°C. After 15 minutes, a 5-ml. portion of appropriately diluted hemicellulase was added to each flask and the contents swirled for 30–45 seconds. After 1 hour, the flasks were cooled for 5 minutes by immersion in cold tap water. A 10-ml. aliquot of 0.1*N* iodine solution was added to each flask and the inside surface was rinsed down with 5 ml. of 8% sodium carbonate. Individual flasks were swirled gently to mix the contents and placed in the dark for 20 minutes. At the end of this time the flasks were taken out and 5 ml. of 2*N* sulfuric acid added at once. The contents were titrated with 0.05*N* sodium thiosulfate, using starch indicator. The titration difference between the blank and the digest was taken as a measure of the hemicellulase activity.

Determination of Phosphorylase Activity and the Effect of Larval Secretions on Phosphorylase Activity. Phosphorylase activity in wheat plant juice was determined using the methods of Cori *et al.* (7) and Sumner (22). One milliliter of a cysteine hydrochloride-disodium beta-glycerophosphate buffer (pH 6.8) was mixed with 40 mg. of glycogen and 1 ml. of 0.064 *M* neutralized glucose-1-phosphate in a 24 × 200-mm. tube graduated at 50 ml. After temperature equilibration in a water bath at 30°C., 0.2 ml. of properly diluted phosphorylase or plant juice was added and allowed to digest the substrate. The activity was stopped at the end of 5 minutes of reaction by the addition of

[5] Unpublished method used by Dr. C. V. Smythe, Rohm and Haas Co., Philadelphia, Pa.

three drops of trichloroacetic acid. The inorganic phosphorus liberated by the reaction was determined using the colorimetric procedure of Sumner (22). The units of phosphorylase were calculated as described by Sumner and Somers (23).

To determine the effect of larval secretions on wheat plant phosphorylase, the following procedure was employed: Small filter papers were placed in sterile Petri dishes and wetted with the plant juice squeezed from plants by means of a mortar and pestle. Dowicide A[6] was added to the juice at the rate of 210 mg/100 ml of juice in order to prevent mold growth. Several active young larvae were placed on one of each pair of filter papers which were wetted with the juice from a given variety. At the end of 24 hours the filter papers were washed thoroughly with 5 ml. of distilled water and aliquots of these solutions were analyzed in duplicate for phosphorylase activity.

Determination of Sugars in Plant Tissue (4, 8, 11). Two grams of wheat leaves which had been dried in a vacuum oven were extracted by refluxing for 2.5 hours in 25 ml. of boiling 85% ethanol. The extract was evaporated to dryness under reduced pressure and the residue dissolved in 3 ml. of distilled water. This solution was deionized by passing it successively through small columns containing 0.5 g. of Dowex 50 and 0.5 g. Duolite A4. Sugars were separated and identified by the paper chromatographic technics of Griffith and Johnson (11). The quantitative analyses of the sugars were carried out by the procedure of Dubois *et al.* (8).

Miscellaneous Technics. The moisture, ash, protein, crude fiber, phosphorus, and silica contents of ground wheat stems were determined by the official methods of the A.O.A.C. (1).

Technics of flame photometry were applied to the determination of certain trace elements in the ash obtained from the stem tissue of different wheat varieties. One gram of plant tissue (dry basis) was ashed in platinum dishes below 600° C. The ash was dissolved in concentrated hydrochloric acid, evaporated to dryness, made up to 25 ml. with 0.01*N* hydrochloric acid, and analyzed, using a Beckman flame photometer.

The pH of the plant juice squeezed from the stems of young wheat plants by means of a Carver press was measured, using a Beckman Model H-2 pH meter.

Results and Discussion

Respiration Activity of Wheat Plants. Respiration rates of stem tissue were obtained for 13 varieties grown under comparable condi-

[6] Dow Chemical Co., Midland, Michigan.

tions and varying in their resistance to the hessian fly.

Table II shows the average rates of respiration expressed as ml/hr/g fresh weight. Varieties are arranged in groups of descending resistance to the fly, to facilitate comparison.

TABLE II

RATES OF RESPIRATION FOR THIRTEEN VARIETIES OF WHEAT VARYING IN THEIR RESISTANCE TO THE HESSIAN FLY

Degree of Resistance	Variety	Oxygen	Carbon Dioxide	Respiratory Quotient[a]
		ml/hr/g	*ml/hr/g*	
1	CI 12855	0.65	0.53	0.82
1	PI 94587 (Durum)	0.65	0.53	0.82
2	CI 12872	0.59	0.55	0.95
2	Illinois No. 1	0.57	0.45	0.79
3	Ponca	0.73	0.71	0.97
3	CI 12854	0.65	0.60	0.92
3	Marquillo	0.58	0.51	0.88
4	Pawnee	0.59	0.56	0.95
4	Kawvale	0.67	0.54	0.81
S	Comanche	0.72	0.65	0.90
S	Tenmarq	0.66	0.55	0.83
S	Oro	0.64	0.61	0.95
S	Mediterranean	0.61	0.48	0.79

[a] Respiratory quotient = carbon dioxide/oxygen.

In order to correlate the respiration data with degree of resistance to the hessian fly, it was assumed that the differences between successive resistant groups were linear (Table II). The correlation coefficients between resistance and oxygen consumption and between resistance and the respiratory quotient were 0.186 and 0.314, respectively. For the 13 varieties used, these correlation coefficient values were considerably less than required for the 5% level (0.553). Thus, although there were significant differences among the respiration activity values for different varieties of wheat, those values were not significantly correlated with degree of resistance.

Hydrogen-Ion Concentration of Juice from Crushed Wheat Plant Tissue. The pH of juice from crushed stem tissue of the different varieties tested ranged from 6.05 to 6.20. There was no relationship between hydrogen-ion concentration of the juice and degree of resistance.

Shearing Resistance of the Wheat Plant Stem. Correlation between resistance to fly attack and resistance to shear was studied, using a series of winter wheats grown under comparable conditions in the greenhouse. Five varieties were grown in seven flats using a completely randomized block design. Each flat contained five rows, one of each of

the tested varieties. Six replicated cuttings were made from each row 1 month after planting, making a total of 42 cuts for each variety. Table III shows the results obtained from several winter wheat varieties. An analysis of variance of these data revealed a significant difference (exceeding the 1% level) between resistance to shear for the different tested varieties. Varieties C. I. 12855, C. I. 12804, and Ponca showed higher resistance to shearing than did Comanche and Tenmarq. These data correlated well with present and past field observations relative to toughness of plants as related to their resistance to infestation. Thus, varieties which have tough sheath and stem tissue are more resistant to the fly. Furthermore, resistance of Pawnee has been found to increase due to the toughening effect of being grown under arid conditions.

TABLE III

RESISTANCE OF WINTER WHEAT PLANT STEMS TO SHEAR

Degree of Resistance	Variety	Lead Shot per 0.001 Inch of Stem Diameter in Seven Replications (Flats)							Average
		g.	g.	g.	g.	g.	g.	g.	g.
1	12855	1.12	0.98	1.26	1.10	1.14	1.04	0.98	1.09
2	12804	0.81	0.93	0.84	0.93	1.05	1.24	1.02	0.99
3	Ponca	0.66	1.03	0.85	0.80	0.89	0.86	1.15	0.89
S	Tenmarq	1.14	0.89	0.77	0.62	0.61	0.61	0.45	0.73
S	Comanche	0.97	0.74	0.43	0.65	0.50	0.54	0.54	0.62

Hemicellulose Content in Wheat Plants and Hemicellulase Activity in Hessian Fly Larvae. One gram of macerated frozen larvae showed a hemicellulase activity equivalent to 45.0 mg. of a commercial enzyme preparation, 45 AP.[7] The presence of hemicellulase in the larvae led logically to a consideration of the amount of hemicellulose which is present in the stems of wheat plants. Hemicellulose was determined in different wheat varieties grown in the absence of infestation at Columbia, Missouri, and Manhattan, Kansas. Samples were collected from both localities in fall and spring. Table IV shows average results obtained from both places.

The correlation coefficient between the hemicellulose content and degree of resistance for all samples tested was 0.928, which is highly significant. These results suggested that there is a direct relationship between the hemicellulose content in the wheat plant stem and degree of resistance to attack by the hessian fly. There was no significant correlation of resistance with crude protein, ash, holocellulose, cellulose, or crude fiber.

[7] Rohm and Haas Co., Philadelphia, Pa.

TABLE IV

AVERAGE ANALYSIS OF WHEAT STEMS OBTAINED FROM VARIETIES GROWN AT COLUMBIA, MISSOURI, AND MANHATTAN, KANSAS

Degree of Resistance	Varieties	Crude[a] Protein	Ash[a]	Holocellulose[b]	Hemicellulose[b]	Cellulose[b,c]
		%	%	%	%	%
	Missouri[d]					
1	Pawnee × 94587	19.9	8.20	50.2	20.2	30.0
2	CI 12804	17.6	7.52	44.2	18.1	26.1
3	Ponca	19.7	7.24	39.7	16.6	23.1
3	CI 12854	20.8	6.98	43.4	15.7	27.7
4	Pawnee	19.5	7.36	44.9	16.0	28.9
S	Tenmarq	21.2	8.37	45.7	12.0	33.7
S	Comanche	18.6	7.55	41.2	13.3	27.9
S	Vigo	20.7	8.06	43.3	11.8	31.5
	Kansas[e]					
1	Pawnee × 94587	14.3	7.99	45.0	20.9	24.1
2	CI 12804	11.3	7.39	43.9	20.2	23.7
3	Ponca	13.2	8.17	46.6	18.4	28.2
3	CI 12854	12.5	7.05	44.6	17.0	27.6
4	Pawnee	10.3	7.33	45.7	18.1	27.6
S	Tenmarq	12.1	6.91	42.8	11.7	31.1
S	Comanche	12.2	7.29	43.9	11.9	32.0
S	Vigo	16.9	9.55	48.9	12.7	36.2

[a] Dry matter basis.
[b] Free from moisture, ash, and protein.
[c] By difference (holocellulose-hemicellulose).
[d] Average of two samples
[e] Average of three samples.

Silica and Trace Mineral Content of Wheat Plant Stems. The silica content of the wheat plant is believed by some (9, 15) to be a factor in the resistance of the wheat plant to attack by the hessian fly. To study this theory, several varieties of wheat plants representing a wide range of resistance were grown at two locations and analyzed for their silica content. At the same time, other trace minerals were determined quantitatively in these plants on the basis that they might be correlated with the degree of resistance. The results of these analyses are recorded in Table V. No significant correlation existed among the amounts of any of these elements and degree of fly resistance.

Relation of Larval Secretions of the Hessian Fly to Phosphorylase Activity and Sugar Content of the Wheat Plant. It has been a common field observation that the central leaves of wheat plants infested with hessian fly develop a dark green color. Leaves of the tillers adjacent to the infested parent plant also show the same symptoms. This is the visual means whereby experienced entomologists distinguish easily between infested and noninfested plants.

The dark green color in plant leaves is regarded as a symptom of phosphorus hunger (12). That this does not appear to be true in

TABLE V

SILICA AND TRACE MINERAL CONTENT OF WHEAT PLANT STEM TISSUE GROWN AT TWO DIFFERENT LOCATIONS AND REPRESENTING SIX VARIETIES[a]

Variety	Silica	Potassium	Sodium	Calcium	Magnesium	Manganese	Copper
	%	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Missouri, Spring, 1954 (Field)							
Pawnee × 94587	1.78	1372	49	75	146	15	10
12804	1.31	1575	41	72	135	16	10
Ponca	1.37	1437	35	70	116	19	13
Pawnee	1.30	1548	34	76	114	15	10
Comanche	1.35	1496	34	79	115	17	10
Tenmarq	1.35	2339	47	122	181	24	13
Kansas, Spring, 1954 (Field)							
Pawnee × 94587	1.91	1260	34	58	78	14	6
12804	1.36	944	25	43	70	12	7
Ponca	1.56	1366	52	61	105	20	11
Pawnee	1.40	1333	44	67	92	18	7
Comanche	1.43	997	29	52	74	14	5
Tenmarq	1.37	1274	27	58	78	15	7
Kansas, Spring, 1954 (Nursery)							
Pawnee × 94587	2.07	1616	46	93	116	13	11
12804	1.97	1631	33	77	104	14	13
Ponca	2.18	1773	38	83	120	16	12
Pawnee	1.89	1329	27	81	92	11	10
Comanche	1.98	1514	29	76	111	12	13
Tenmarq	1.49	1300	22	76	86	11	10

[a] Dry matter basis.

wheat infested with hessian fly was shown by the data for total phosphorus determined in infested and noninfested wheat plants sampled during a period of 9 days after initial infestation. The infested plants actually contained 7 to 28% more total phosphorus than healthy plants. Since these plants were grown in the greenhouse, in soil which was mixed thoroughly before being placed in the different flats, the results indicated that phosphorus hunger symptoms of hessian fly-infested plants are not due to soil conditions but may be due to a factor conferred on the plant by the larvae.

Hambidge (12) and Onslow (16) have stated that the accumulation of photosynthetic products in plant cells intensifies the green color of plant leaves and that these products may be sugars. In the present study it was assumed that sugar might accumulate in infested plants if the phosphorylase enzyme system was inactivated or inhibited. To test this hypothesis, the juices expressed from the stems of different varieties of wheat were used as liquid media for active hessian fly larvae supported on filter paper which acted as a wick for the

plant juice. The data recorded in Table VI suggest that the larvae secreted something which partially inactivated or inhibited the phosphorylase present in the juice from wheat plants. The inactivation or inhibition was apparent for both resistant and susceptible varieties.

TABLE VI

EFFECT OF LARVAL SECRETIONS OF HESSIAN FLY ON WHEAT PLANT PHOSPHORYLASE ACTIVITY

Material Used	Phosphorylase Units[a] After 24 Hours of Incubation	Inactivation
		%
Resistant varieties		
Ponca juice	45.0	
Ponca juice + larvae	22.0	51.0
12804 juice	14.2	
12804 juice + larvae	12.9	8.9
Durum juice	70.5	
Durum juice + larvae	40.5	42.5
Susceptible varieties		
Pawnee juice	15.6	
Pawnee juice + larvae	12.9	16.6
Tenmarq juice	93.5	
Tenmarq juice + larvae	79.0	15.5

[a] According to Sumner and Somers (23).

TABLE VII

SUGAR CONTENT OF COMANCHE WHEAT PLANTS AT DIFFERENT STAGES OF INFESTATION

Sugars	Sugar Content of Dry Material				
	Days After Infestation				
	1	3	5	8	10
	mg/100 g	*mg/100 g*	*mg/100 g*	*mg/100 g*	*mg/100 g*
Sucrose					
Infested	180	619	658	727	1051
Noninfested	280	425	395	412	566
Glucose					
Infested	307	524	589	677	937
Noninfested	188	266	325	430	354
Fructose					
Infested	149	305	406	475	552
Noninfested	118	260	259	307	301
Total sugars					
Infested	636	1448	1653	1879	2539
Noninfested	586	951	979	1149	1221
Percent increase in total sugars	9	52	69	64	108

Sucrose, glucose, and fructose were determined in leaves of infested and noninfested wheat plants during a 9-day period after infestation started. The data in Table VII show that the total amount of sugars increased from 636 mg. after 1 day of infestation to 2539 mg. per 100 g. of dry material during the 9 days of infestation. The total amount of sugars in infested plants was essentially double that in healthy plants. The reasons for this increase in sugar in infested plants are not clear. The increase may be due to inhibition of the plant phosphorylase by insect secretions, or it may be due to a greater concentration of chlorophylls in certain leaves of infested plants than in leaves of healthy plants. The accumulation of sugar in infested plants may be a side effect rather than the cause of greener leaves.

Conclusions

It is believed, on the basis of the information obtained in this study, that the larvae start to feed by secreting first a hemicellulase, which permits the insect to obtain the plant juices by means of a sucking action. During this process, the larvae secrete something which blocks the activity of the plant phosphorylase.

Larvae migrate to the feeding area on all varieties but are unable to molt in the confining space on resistant plants. It is proposed that in susceptible varieties the plant tissues yield easily, thus affording a place for the larvae to grow. In resistant varieties the tissues are tough enough, due to the presence of a large amount of hemicellulose, to prevent normal feeding or to restrict the space below that necessary for normal growth and development of the larvae. Resistance to the insect may be associated with inability of the insect to obtain juices from the plant.

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STUDIES ON THE INCORPORATION OF NONFAT MILK SOLIDS IN WHOLE WHEAT BREAD
II. MIXOGRAMS[1]

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ABSTRACT

Mixograms made on unfermented whole wheat flour doughs showed that addition of heat-treated spray-dried nonfat milk solids to the other ingredients increased dough development time, decreased height and width of curves, and decreased somewhat the dough weakening angle. The area under the mixogram curves on fermented doughs was decreased by addition of milk solids. All of the factors varied in degree of change, due to the type of flour used. Nonfat milk solids increased dough development time in both the mixograph and in the baking tests. No property of the mixograms appeared to correlate with loaf volumes of breads.

In the literature, most of the mixograms made with the addition of nonfat milk solids have been for the purpose of comparing the effects produced by milk solids of poor quality and by milk solids of good quality. Few studies have shown the effects of varying amounts of heat-treated, nonfat milk solids in flour dough. Johnson and Swanson (3), in a study on the effect of fermentation, rest periods, and formula ingredients on mixogram patterns, included dry milk solids. These workers used 0, 2, 6, and 10% milk, which replaced part of the flour. They did not describe the sample of milk or the flour used.

In the present study, mixograms were made from some of the same flours that were used in the baking tests on the incorporation of nonfat milk solids in whole wheat bread (4). The mixograms on the doughs, which contained from 0 to 22% nonfat milk solids, are reported here and compared with previous baking tests.

Materials and Methods

Materials. Eleven samples of flour were used: eight known, single-variety whole wheat flours; two commercial whole wheat flours, S and X; and one commercial blend of spring and winter wheat, 95% patent flour, E. The sample of spray-dried nonfat milk solids used in both baking and mixograph tests was a commercial milk especially prepared for bread baking, which had shown good water-absorption and baking qualities in this laboratory. The flour samples and the results of the baking test are described in a previous paper (4).

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Methods. Mixogram curves were made on a National recording micro mixer, using 25 g. of flour on a 14% moisture basis. Other dough ingredients were added according to the following formula: flour, 100%; water, 59–65% depending upon the flour; yeast, 2%; sugar, 4%; salt, 2%; shortening, 2%; nonfat milk solids, 0–22%. Milk solids were added at the levels of 0, 6, 12, 16, 18, and 22%, with 1 g. of water for each g. of milk. The mixer was run for 10–24 minutes at a spring setting of 9. The head speed of the mixer was 86 r.p.m. The method of Swanson and Johnson (8) was used to evaluate the mixograms made from the dough ingredients. Measurements made were the time, in minutes, to reach the maximum curve height; the magnitude of this height in units; dough development angle; dough tolerance angle; and dough weakening angle.

Mixograms were also made on 30-g. units of the fermented doughs after 135 minutes of fermentation. These doughs were scaled from those used in the baking tests (4). The mixograph was run for 8 minutes at a spring tension of 8. The mixograms were compared by measuring the area beneath the curves by means of a planimeter, as described by Morris, Bode, and Heizer (5).

Results and Discussion

In making the mixograms, the weights of flour, salt, yeast, and sugar were kept constant, and the milk solids and additional water were added to those ingredients, just as was done in mixing the doughs for the baking tests. This resulted in increasing weights of dough in the mixer as the amount of milk solids was increased. Without milk solids, the total dough weight in the mixer was from 41.7 to 43.8 g., depending upon the moisture absorption of the flour. Then, for example, if the basic ingredients weighed 41.7 g. with no milk, the dough weight with 6% added milk solids (flour basis) was 44.7 g., with 12% milk solids, 47.7 g., until with 22% milk, the dough weight was 52.7 g.

Stamberg and Merritt (6) showed the importance of controlling the amount of dough to ensure accurate use of the farinograph, particularly when interpreting curve height. Therefore, to test which of the changes following the addition of milk solids was due to the milk itself, and which might be due to the increase in dough weights, a series of curves was run on doughs containing no milk and 22% milk. The ratio of the ingredients was kept the same as in the original tests, but the dough weights were adjusted to 42.8 g. in one case (the weight of the no-milk dough), and to 53.8 g. in the other (the weight of the 22% milk dough). Wichita flour was used.

Measurements of these curves are given in Table I. In measuring the angles of development, tolerance, and weakening, lines were drawn through the center of the curves, corresponding to 1½ minutes on each side of the point of highest development. Some difficulty was experienced in judging these lines when the milk solids were added. With the larger amounts of milk, several minutes elapsed before the dough began to develop. Once the development began, the curves resembled those made with smaller amounts of milk. Compare the mixograms made from 22% milk solids with those from 6% milk in Figs. 1 and 2. In addition, the milk produced curves which tended to flatten on top, with no well-defined peaks.

TABLE I

EFFECT OF NONFAT MILK SOLIDS AND OF THE WEIGHT OF DOUGH IN THE MIXER ON THE MIXOGRAM PATTERNS OF DOUGH INGREDIENTS MADE WITH WICHITA WHOLE WHEAT FLOUR[a]

Nonfat Milk Solids, Flour Basis	Weight of Dough	Dough Development Time	Mixogram Characteristics				
			Height	Width	Development Angle	Tolerance Angle	Weakening Angle
%		*Minutes*	*Units*	*Units*	*Degrees*	*Degrees*	*Degrees*
0	42.8 g.	6.0	42	12	24	150	6
22		18.8	24	5	8	172	0
0	53.8 g.	4.8	70	33	26	142	12
22		18.4	37	7	18	160	2

[a] Each value represents the average of two mixograms.

The amount of dough did not appreciably affect the time required to develop either the no-milk or the milk doughs (Table I). Thus the increase in dough development time appears to be the result of the addition of the milk solids and added water, and to be independent of the increase in dough weight. With the no-milk doughs, the extra weight increased the height and the width of the curves, and increased the weakening angle. The angle of tolerance was somewhat decreased, and the angle of development very slightly increased. Similar changes were noted in the milk doughs except that the width of the curve and the weakening angle were only slightly increased, while the angle of development was considerably increased.

The effect of the milk and added water at each given dough weight was to increase the mixing time and to decrease the height and width of the curve, the angle of development, and the weakening angle. The angle of tolerance was increased. The effect of the increase in dough weight was taken into consideration in the interpretation of the mixograms in which the dough weight was not kept constant.

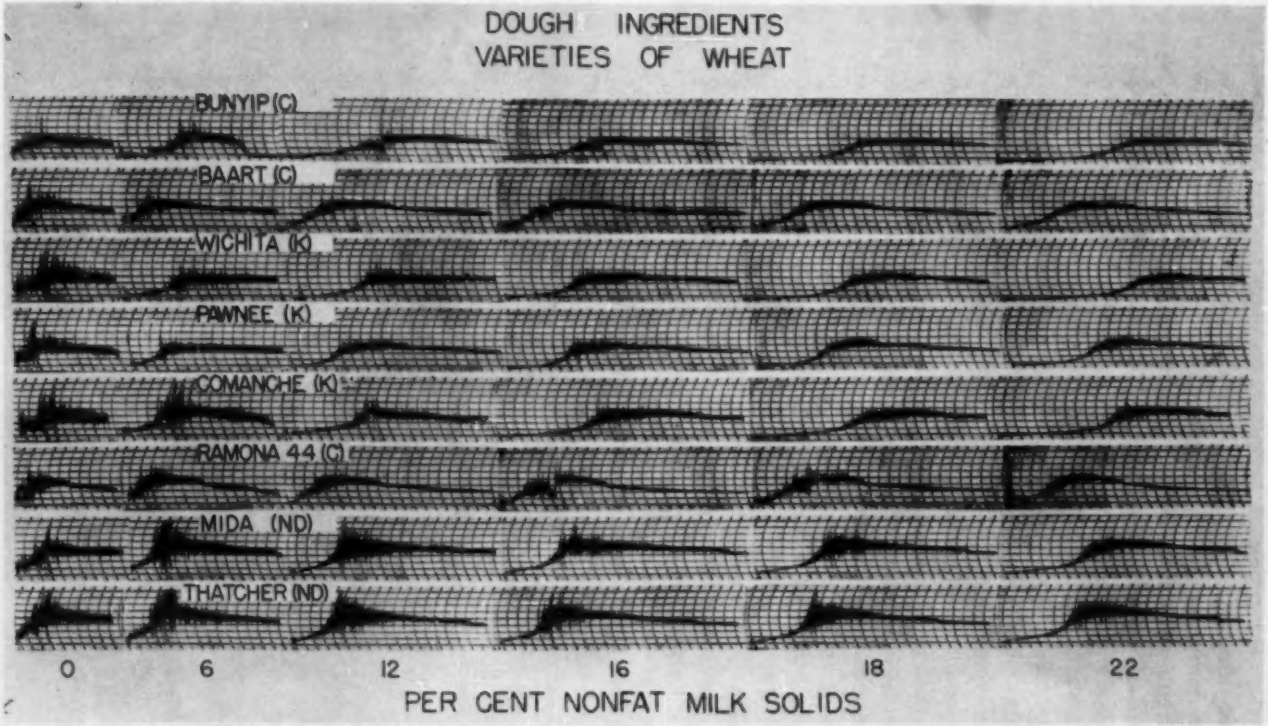

Fig. 1. Mixograms obtained from eight known, single-variety, whole wheat flours with varying amounts of nonfat milk solids. The curves were obtained from unfermented mixes and contained all of the baking formula ingredients. Bunyip and Baart are wheat varieties Bunyip 41 and Baart 38. C, K, and ND represent California, Kansas, and North Dakota, respectively, the states where the wheat was grown.

Water Absorption of Nonfat Milk Solids. In this work one part by weight of water was added with each part of nonfat milk solids, a water absorption of 100% for the milk. This is somewhat higher than that frequently encountered in published literature, but is the amount recommended for a good baking milk (1). This amount of water was also used in the baking tests with all of the whole wheat flours, except that a slight reduction was necessary with Bunyip 41, Baart 38, and Ramona 44 when 18 and 22% milk solids were added, and with Ramona 44 with 16% milk.

In evaluating mixogram curves, Swanson and Andrews (7) and Harris *et al.* (2) found that the effect of increasing the water absorption of no-milk doughs was to increase the time of development and to decrease the height and, to a lesser extent, the width of the curves. From an inspection of the mixograms on flour-water doughs presented by Swanson and Andrews, an increase in the water absorption of 24% increased the time of development for one flour from 2¾ minutes to 7½ minutes, and for a second flour from 1 minute to 1½ minutes. This same increase in water decreased the curve height of both flours from approximately 80 to 55 units. The doughs which were stiff at 58% absorption gradually changed to very soft in various grades of stiffness as the water was increased. The authors described the mixture made with 90% absorption as more like a batter than a dough.

TABLE II

EFFECT OF NONFAT MILK SOLIDS ON DOUGH DEVELOPMENT TIME IN WHOLE WHEAT DOUGHS AS DETERMINED FROM MIXOGRAM CURVES

FLOUR	PROTEIN[a] N × 5.7	PERCENT NONFAT MILK SOLIDS					
		0	6	12	16	18	22
		Dough Development Time					
	%	*min.*	*min.*	*min.*	*min.*	*min.*	*min.*
Bunyip 41	10.4	3.7	7.8	12.8	11.0	13.0	14.3
Baart 38	11.3	3.1	3.7	5.6	6.6	7.5	7.4
Wichita	11.3	3.8	6.1	7.7	11.9	13.7	16.0
Pawnee	12.1	2.9	4.5	7.7	8.1	9.5	10.1
Comanche	12.3	3.5	5.7	8.8	11.8	13.7	14.4
Ramona	12.4	2.7	3.1	4.8	5.9	6.2	7.0
Mida	14.8	3.4	3.9	5.6	7.1	8.2	10.7
Thatcher	15.9	3.1	4.0	5.0	6.5	7.9	8.4
Whole wheat S	14.5	3.8	4.5	6.9	8.8	10.3	11.4
Whole wheat X	14.8	5.1	7.0	8.6	10.2	11.0	14.1
95% Patent, E	12.1	3.3	3.5	4.0	4.9	6.0	7.2
Range		2.7–5.1	3.1–7.8	4.0–12.8	4.9–11.9	6.0–13.7	7.0–16.0
Average		3.5	4.9	7.0	8.4	9.7	11.0

[a] 14% moisture basis.

In the present study the greatest increase in added water was 22%, used with the 22% level of nonfat milk solids. All of the doughs were of approximately the same consistency as determined by the appearance and feel of the dough, and they performed well in the baking tests (4). Objective measurements of consistency were not made. It is possible that some of the effects noted in the present study were due to the effect of added water, although no systematic attempts were made to separate the effects of the milk from those of the water. However, water must be added with the milk to produce a satisfactory dough for baking. Hereafter when reference is made to the effect of milk, it implies the effect of the added water as well.

Dough Development Time. Figure 1 shows the influence of nonfat milk solids on the mixogram patterns made with whole wheat flours of known variety, using all the dough ingredients. Similar data for the commercial flours are shown in Fig. 2. Measurements of the dough development time for these flours are given in Table II. In all cases the time required to develop the dough (as determined by the highest point on the mixogram) increased as the amount of milk solids was increased, but the amount of increase differed with the type of flour. The whole wheats Baart 38 and Ramona 44, and flour E, a 95% patent, showed the least effect – 22% milk solids increasing the development time about two and one-half times that of 0% milk solids. Wichita and Comanche showed the greatest increase in development time—about four times that of no-milk doughs. This increase in the time required to develop the dough to its optimum consistency was also shown in the baking tests (4). However, in the baking tests the speed of mixing was slower than in the mixograph, and a proportionately longer time was required to develop the doughs as the amount of milk solids was increased—from 5.7 to 7.6 times longer for 22% milk solids than for 0% milk in these same whole wheat flours. When milk solids were added, the flour with the shortest dough development time in the mixograms, Ramona, had the shortest development time in the baking tests, while Wichita had the longest development times both in mixograms and in baking tests. This relationship between mixing time and speed was noted by Swanson and Bayfield (9). They observed that at all speeds of all machines, optimum mixing time increased with increasing milk solids, but the increase was greater at low speeds than at high. Milk solids up to 12% were used. These workers also noted that type of flour influenced mixing times. Although this increase in dough development time has been recorded by a few investigators, many of the studies on the incorporation of nonfat milk solids in

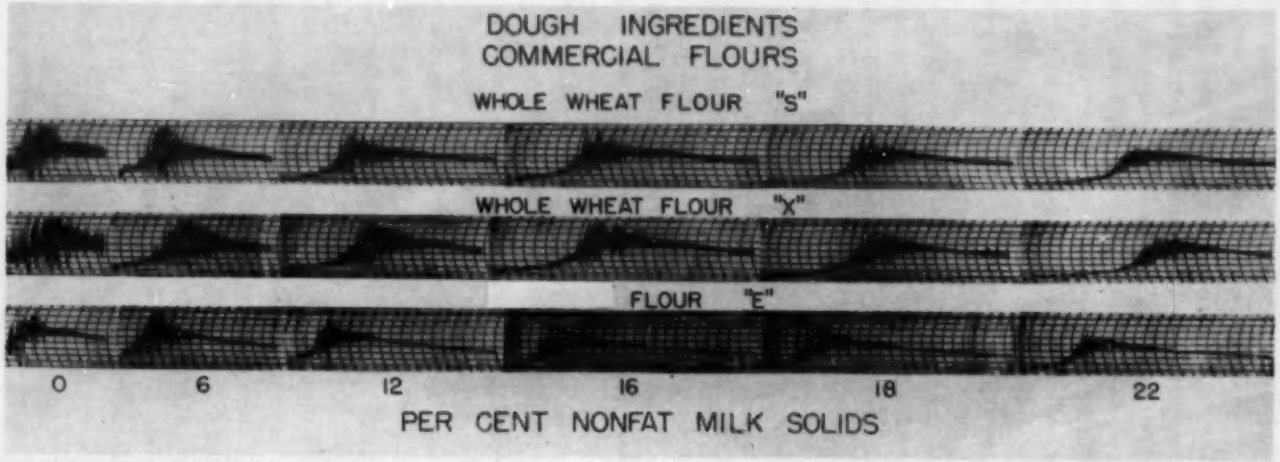

Fig. 2. Mixograms obtained from two commercial whole wheat flours and one 95% patent, with varying amounts of nonfat milk solids. The curves were obtained from unfermented mixes and contained all of the baking formula ingredients.

bread doughs make no mention of either mixing time or speed, or both. This may be due to use of high-speed mixers or to use of lower quantities of milk solids, such as 4 or 6%, where the extra mixing time is not so notable as with larger quantities of milk.

Height of Curves. With all flours, the height of the curves remained almost the same when the amount of milk solids was increased by adding the milk to a constant quantity of other dough ingredients. The lack of change in the curve height in this series is probably a balance between an increase due to the greater weight of dough, and a decrease due to the larger amount of milk solids, since Table I shows that an increase in weight of the dough increased the curve height whether milk solids were present or not, and that when dough weight was kept constant, milk solids decreased the curve height.

Width of Curves. The width of the curves was somewhat decreased with all flours when the amount of nonfat milk solids was increased, and much less swing was observed in the first portion of the curves. Had the dough weights in the mixer been equalized, addition of milk solids would very likely have decreased the curve width even more. Table I shows that an increase in dough weight caused an increase in curve width, the increase being greater in no-milk doughs than in those with 22% milk solids. When dough weight was kept constant, however, the effect of addition of milk solids was to decrease curve width.

This decrease in curve width does not agree with the observations of Johnson and Swanson (3). They added 0, 2, 6, and 10% dry milk solids to flour-water doughs, with and without fermentation. The milk solids replaced part of the flour, so that the weight of the total solids remained constant. The mixograms were made at 89 r.p.m. with a spring setting of 9, except for the fermented doughs, at a spring setting of 11. These workers stated that results showed that the effect of milk was mostly on band width, which increased in all the mixograms, and that the mixograms obtained after 3 hours of rest and after 3 hours of fermentation were notably similar, but markedly different from those obtained from no-rest doughs. They did not describe the sample of milk or the flour used, nor did they record the water absorption of the milk. It is possible that the latter was less than that used in the present study.

Dough Development, Tolerance, and Weakening Angles. The dough weakening angle tended to decrease with the addition of milk solids. With no milk, angles ranged from 4° to 9°, an average of 7°. With 12% milk solids, angles ranged from 0° to 5°, an average of 2.5°. Little change from this latter value was observed with addition of 16,

18, or 22% milk. The angle of development tended to decrease somewhat with addition of milk solids, and the angle of tolerance tended to increase. However, there was considerable variation both among flours and with different amounts of milk, so that the results of these measurements are inconclusive. It has been noted previously that difficulty was encountered in judging the position of the angles on the mixograms, and results between operators, as well as duplicate measurements by the same operator, did not always agree.

Mixograms on Fermented Doughs. Table III shows the influence of nonfat milk solids on the curve area under the mixograms of the fermented doughs. Figure 3 shows mixograms from six of the flours. When compared with no-milk doughs, curve areas of 6% milk doughs decreased. With most of the variety flours, another slight decrease was obtained with 12% milk doughs. Further additions of milk produced little change in curve area in most cases. With commercial whole wheat flours S and X, little difference was shown by different amounts of milk solids.

The width of the curves and the to-and-fro swing of the pen decreased as the amount of milk solids was increased. This, and the decrease in curve area, is evidence that the dough offers less resistance to the moving pins. Johnson and Swanson (3), however, had observed an increase in curve width in mixograms on fermented doughs when up to 10% dry milk solids had replaced part of the flour.

TABLE III

EFFECT OF NONFAT MILK SOLIDS IN FERMENTED WHOLE WHEAT DOUGHS AS MEASURED BY THE CURVE AREA UNDER THE MIXOGRAMS

FLOUR	PERCENT NONFAT MILK SOLIDS					
	0	6	12	16	18	22
	Area under the Mixogram after 7 Minutes in the Mixograph					
	sq. cm.	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*
Bunyip 41	40	36	32	..	..	29
Baart 38	45	42	36	38	35	37
Wichita	41	35	32	28	32	
Pawnee	42	35	32	32	30	27
Comanche	41	33	31	28	26	15
Ramona 44	51	45	42	44	41	37
Mida	54	43	43	39	38	39
Thatcher	55	47	45	43	43	42
Whole wheat S	48	39	40	40	40	37
Whole wheat X	48	44	46	43	44	46
Range	40–55	33–47	31–46	28–44	26–44	15–46
Average	46.5	39.9	37.9	37.2	36.6	34.3

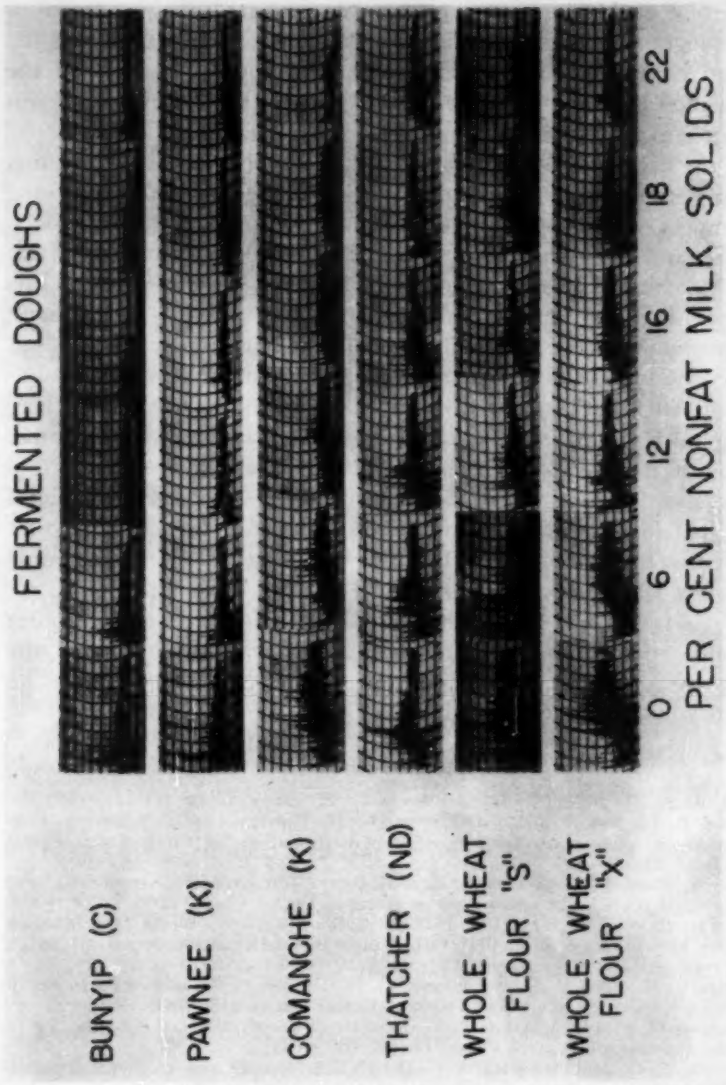

Fig. 3. Mixograms from fermented doughs containing varying amounts of nonfat milk solids. Bunyip is the wheat variety Bunyip 41. C, K, and ND represent California, Kansas, and North Dakota, respectively, the states where the wheat was grown.

Comparison of Mixograms with Baking Tests. Results of the mixograms on dough ingredients showed that addition of nonfat milk solids progressively increased dough development time, decreased height and width of the curves, and somewhat decreased the dough weakening angle, although the degree of change varied in all these factors because

of the type of flour used. From these data, and from the curve area of the mixograms on the fermented doughs, it would appear that as the amount of milk solids increased, the time required to develop the dough increased, and that when it did develop, the dough offered less resistance to the moving pins.

In the baking tests (4), using the 2% yeast formula, the largest loaf volumes from the different flours were obtained with the following percentages of milk: 0% nonfat milk solids, Ramona; 6%, Bunyip 41, Pawnee, and commercial flour S; 12%, Baart 38, Wichita, and Mida; 16%, Comanche. Loaf volumes with Thatcher remained almost the same with 0 to 22% milk solids, and commercial flour X had the largest volumes at 12 to 22% milk solids. Dough development time was affected by type of flour, and increased as the amount of milk solids was increased, both in the mixograph and in the baking tests. There appears to be no property of the mixograms that correlated with loaf volumes of the breads when nonfat milk solids were incorporated into the doughs. Ramona wheat, for example, did not tolerate milk solids when the dough was baked into bread, but gave increasingly lower volumes as the amount of milk solids was increased. It was the only flour used which gave such low bread volumes with milk solids, yet there was nothing in the mixograms to distinguish it from the other flour-milk mixograms. In most cases, the decreased resistance of the dough up to a certain point appeared to favor, rather than hinder, increased volume in the finished loaf.

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CHOPIN ALVEOGRAPH STUDIES. I. DOUGH RESISTANCE AT CONSTANT SAMPLE DEFORMATION[1]

I. HLYNKA AND F. W. BARTH

ABSTRACT

Studies with the Chopin Alveograph served to develop mathematical background for the measurement of resistance of the dough bubble to expansion at constant sample deformation. Data are provided on the relations between the bubble volume, surface area, and wall thickness and their time-dependence in the process of bubble inflation in the alveograph. The pressure in the alveograph bubble is recalculated to dough "resistance" at constant wall thickness of the bubble. "Resistance" is considered to be a fundamenetal measurement analogous to that of load at constant sample elongation which was developed earlier for the extensograph.

The Chopin Alveograph is an empirically designed dough-testing instrument that inflates a thin sheet of dough into a bubble by means of air pressure. The behavior of the dough is summarized by recording, on a kymograph chart, the air pressure within the bubble during the entire course of inflation until rupture. This apparatus was first described by its inventor (2, 3, 4) and subsequently by others (1, 7, 11, 12), and has been extensively used in many cereal laboratories.

The alveograph is an imitative instrument; it simulates the inflation of bubbles in dough by the carbon dioxide produced by yeast fermentation. But it is also a stress-strain type of instrument; the force is supplied as air pressure and the extension of the sample is two-dimensional. It is thus somewhat analogous to the Brabender Extensograph in which, however, the extension is in one dimension only, and a mechanical force is applied to the sample directly.

A considerable advance has been made in extending the application of the extensograph to more fundamental rheological studies by adopting resistance at constant sample deformation as a basic measurement. This basis in turn led to the development of the method of structural relaxation (5, 6) which has proved to be a useful new tool in investigating the enigma of dough structure and dough behavior. A detailed summary of the structural relaxation method has been recently published (8).

The progress made with the extensograph suggested a reexamination of the potentialities of the alveograph, especially with a view to

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adapting it for structural relaxation studies based on measurements made at constant sample deformation. As a first step it was necessary to make a systematic mathematical analysis of the dough bubble, and of the inflation process, and finally to examine the significance of the pressure recorded on an alveogram. These data made it possible to obtain a fundamental basis for comparison of alveograms at constant sample deformation. The results of the mathematical analysis are summarized in this paper. The next step was to develop, on the basis of resistance of dough at constant sample deformation, a procedure for the application of the alveograph to structural relaxation study of dough. This work is presented in a companion paper (9).

Experimental

Elucidation of the mathematical background of the processes involved during the expansion of the dough bubble involves studying: first, the geometry of the alveograph bubble with reference to height, volume, area, and bubble wall thickness; second, the time-dependent processes, i.e., the change of volume, area, and thickness with time; and finally, the relation of these variables to the pressure changes which take place during bubble expansion. The data in this section are presented in that order. No distinction is made between data obtained by mathematical analysis and data obtained as a result of test procedures on dough; both are considered as "experimental" data.

Geometry of the Alveograph Bubble. The alveograph bubble starts as a flat disk of dough (5.5 cm. in diameter and 0.25 cm. thick), the

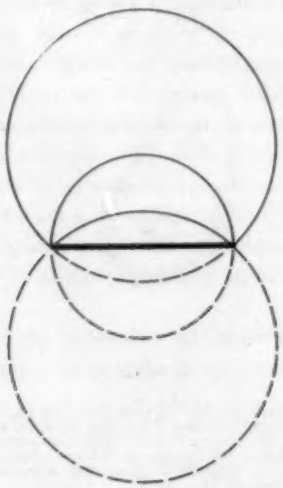

Fig. 1. Inflation of the dough bubble represented as spherical sections.

dimensions of which are fixed by the dimensions of the apparatus. As air is injected underneath the dough membrane, the bubble closely approximates the shape of a sphere in accordance with minimum energy considerations.

Figure 1 is an idealized representation of what happens as the size of the dough bubble increases. At first the bubble is a small section of a large sphere with the annulus of the alveograph as the base of the section. The large section of the sphere is missing and is shown as a dotted line below the base. As the bubble increases in size the base of the spherical section remains constant, but the sphere of which the section is a part becomes smaller and smaller. Eventually the bubble forms a half-sphere with the diameter of the annulus as the diameter of the sphere. As the size of the dough bubble increases beyond the half-sphere, it becomes a large section of a large sphere with the small section missing. Beyond this the bubble becomes an increasingly larger section of an increasingly larger sphere, until the bubble finally ruptures (see column 2 of Table I).

On the basis of the foregoing idealized representation of the alveograph bubble, as it increases in size, it is possible to calculate its volume, area, thickness, etc. Table I summarizes these data.

The height of the alveograph bubble is shown in the first column. These values were arbitrarily selected to cover the range of dough

TABLE I

CALCULATED DATA ON ALVEOGRAPH BUBBLES OF VARYING SIZE

Height of Bubble	Radius of Sphere	Area of Bubble Section	Volume of Bubble Section	Thickness of Bubble Wall
cm.	*cm.*	*cm.*^{2}	*cm.*^{3}	*mm.*
0.60	6.65	25.0	7.2	2.38
0.70	5.44	25.4	8.4	2.34
0.80	5.16	25.9	9.9	2.29
1.00	4.31	27.0	12.4	2.20
1.20	3.78	28.4	15.1	2.09
1.50	3.29	31.7	19.6	1.87
2.00	2.91	38.0	27.9	1.56
2.50	2.77	43.5	37.8	1.37
3.04	2.77	52.9	51.5	1.12
3.82	2.91	68.5	75.6	0.87
5.08	3.29	104.3	129.1	0.57
6.36	3.78	143.7	211.0	0.41
7.62	4.31	206.5	323.9	0.29
9.52	5.16	301.5	567.0	0.20
10.18	5.44	339.8	676.6	0.18
12.17	6.65	523.8	1228.0	0.11

bubbles encountered in using the alveograph. It should be noted at this point, that for structural relaxation studies which are described elsewhere (9) the capacity of the air buret of the alveograph was increased from 900 cc. to 2000 cc. by installing a larger buret.

The second column of Table I gives the radii of spheres corresponding to the heights of bubbles given in the previous column. The radius is calculated as the hypotenuse of a right-angled triangle, of which one side is the radius of the base of the bubble section and the other side is the difference between the radius of the sphere and the height of the bubble section. The radii of spheres for bubble sections larger than a half-sphere are obtained indirectly from calculations for the small spherical sections representing the portion missing from the bubble. The remaining data in Table I were calculated, according to standard formulas (10), from the height of the spherical section and the radius of the sphere of which the section forms a part. The areas and the volumes of the spherical sections are shown in the third and fourth columns. The data for sections smaller than a half-sphere were calculated directly from the formulas, and those for the larger sections were obtained as the difference between the total sphere and the complementary small section. Finally, the last column gives the thickness of the dough bubbles. This was obtained by dividing the volume of the original dough membrane, which is 5.49 $cm.^3$, by the area of the dough bubble when inflated to given dimensions.

The dimensions of an alveograph bubble are, of course, an approximation to the values calculated for ideal spherical sections. However, the apparatus uses a thin sheet of dough and inflates the bubble rapidly, using very low air pressure. Under these conditions there is very little error resulting from the dough's pulling out at the base of the sphere. Moreover, observation of the bubbles formed in numerous tests indicates that the majority of them are symmetrical, which implies a uniform thickness of the dough membrane. Occasionally there seems to be a gradation in membrane thickness from the top towards the base of the bubble, and once in a while irregularly shaped bubbles are inflated. The pressure recorded on an alveogram, however, gives a sort of a mean value over the range of dough membrane thickness in any one bubble. All in all, the deviations of the dimensions of an actual bubble from the dimensions calculated for the spherical section appear to be small enough to justify the use of theoretical calculations.

Time-Dependent Variables in the Inflation of the Alveograph Bubble. The time-dependent variables involved in the process of inflating the dough bubble will be discussed next. The data are summarized in Table II.

TABLE II

TIME-DEPENDENCE OF ALVEOGRAM AND DOUGH BUBBLE DIMENSIONS

Time	Distance on Alveogram	Volume of Bubble	Area of Bubble	Thickness of Bubble Wall
sec.	*cm.*	*cm.*^{3}	*cm.*^{2}	*mm.*
0.2	0.12	5	28	2.13
0.5	0.30	12	32	1.86
1.0	0.59	25	42	1.41
2.0	1.10	50	57	1.04
3.0	2.36	75	73	0.81
4.0	2.95	100	87	0.68
5.0	4.22	125	103	0.58
7.0	1.77	175	132	0.45
9.0	5.33	225	157	0.38
10.0	5.89	250	170	0.35
12.0	7.26	300	190	0.31
14.0	8.46	350	218	0.27
15.0	8.83	375	228	0.26

Although time is the basic independent variable (first column), it is convenient in practice to use the distance along the base of the alveogram chart as an indirect measure of time. The data in the second column of Table II were calculated directly from the measured rate of chart travel of 0.589 cm. per second.

The data summarized in the third column show the rate of water flow into the air buret and hence the rate of increase of the volume of the dough bubble. Experimentally these data were obtained as follows. First, the over-all flow time of 25.1 ml. per second was established in the range recommended by the manual of instructions supplied with the instrument. This rate is slightly below the rate specified in the manual, but was the maximum flow that could be obtained. Then flow times for each 100 ml. were determined, using a dough membrane in the apparatus. There is a slight retardation of flow with a dough membrane. A calibration curve was then constructed for the apparatus with the dough membrane, and this curve was used to evaluate the rate of air displacement in the air buret at any time.

An assumption is made that the amount of air displaced by the water is equal to the volume of the bubble. A small error results from the compression of the air, but is considered negligible. It can be readily evaluated as follows. The maximum pressure corresponding to the vertical range of the alveograph chart was equivalent to 160 mm. water or 11.7 mm. mercury. At atmospheric pressure of 760 mm. mercury, it may therefore be calculated that reduction in volume due to

compression would be by about 1.5% for the maximum case and considerably less for the average case.

The next step is to evaluate, from the time and volume data in the first three columns of Table II, the time-dependence of the area and the thickness of the bubble during inflation. The area and thickness of the dough bubble, which are given in the last two columns of the table, are reciprocally related to one another. From the point of view of dough properties it is preferable to use thickness, since the resistance or restraining force of the dough is directly related to the thickness. For certain graphical representations, however, the area of the dough bubble is preferable.

It will be noted that the data in Table II were not taken beyond the time of 15 seconds and a bubble volume of 375 cc., and thus include approximately the first third of the full horizontal range of the alveograph chart. The reason for this is that in attempting to provide a common basis of comparison of alveograms one is necessarily limited by the dimensions of the smallest alveogram. Moreover, the same data, and perhaps more reliable, are obtained from the early part of the alveogram as from the subsequent portion.

Analysis of the Alveogram. With the data presented above, the significance of the pressure recorded on an alveogram can now be examined, and the conditions necessary for constant sample deformation with the alveograph can be defined.

An alveogram is a record of the pressure within the bubble as it is inflated until the bubble bursts. In a study of dough properties, however, it is more instructive to focus attention on the resistive force of the bubble wall in opposing the inflating pressure. The resistive force of the dough is clearly a function of two distinct factors: some dough property related to its tensile strength, and the thickness of the wall of the dough bubble. It is the first of these two factors which is of primary interest. It can be separated out mathematically by dividing the resistive force (i.e., alveogram pressure) at any time by the thickness of the bubble wall at that time. This resistive force of the dough referred to a constant thickness of the dough wall is termed the alveogram "resistance" of the dough, and is shown to be a fundamental measurement.

Figure 2 shows graphically the relation between the alveogram pressure, the resistance, and the area of the dough bubble. (Here area was used instead of the wall thickness, because it possessed a definite advantage for graphing). A sample alveogram is shown at the top. The conditions under which it was obtained are not relevant here, as another alveogram would yield similar information. More extensive

information, however, is presented in the companion paper (9).

Figure 2, center, shows the alveogram replotted in terms of dough "resistance." The pressures recorded on the alveogram were recalculated to a bubble wall thickness of 1 mm. The pressure is in terms of cm. of water. For this purpose the alveograph manometer was calibrated by connecting it directly with a water manometer. The calibra-

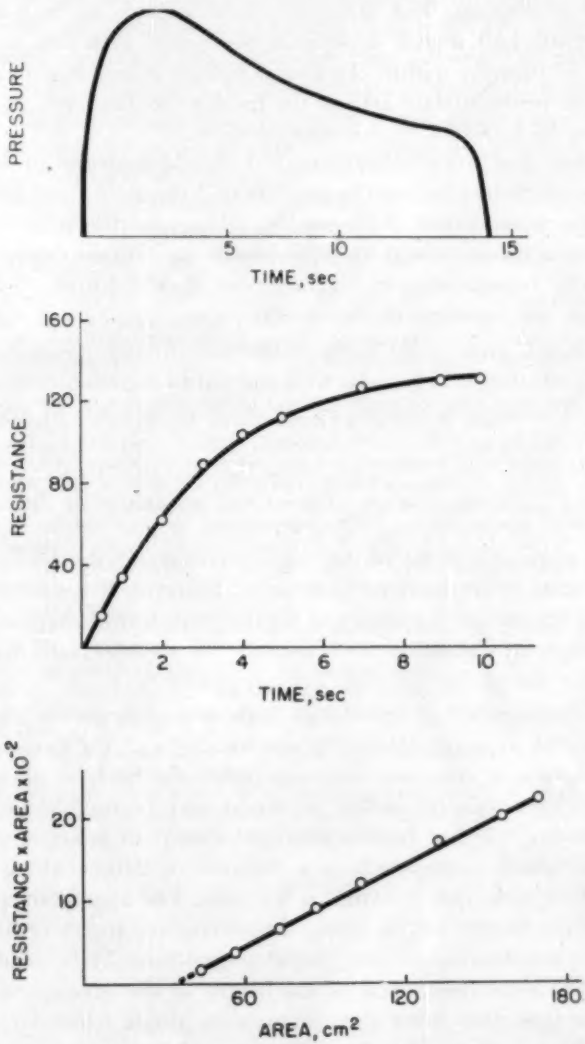

Fig. 2. The alveogram and its transformations.

tion chart that was used may be represented by

$$y = 1.5\,x + 0.03$$

where y is the manometer pressure in cm. water and x is the height of the alveogram, also in cm.

An interesting characteristic of the "resistance"-time curve is the complete absence of any feature corresponding to the maximum on an ordinary alveogram. The maximum thus appears to be a fortuitous characteristic and merely represents the point at which the rate of increase in pressure within the dough bubble is just equal to the rate of increase in the surface area of the bubble. Beyond this, little significance can be attached to this measurement.

Another and even more revealing transformation of the alveogram was made by plotting the product of "resistance" and area against area. This transformed the complex alveogram shown at the top of Fig. 2 into a simple straight line, shown at the bottom. More extensive tests of this relationship with alveograms obtained under a variety of conditions are reported in the second paper.

The significance of the slope of the straight line (resistance × area vs. area plot) may best be seen with the aid of elementary dimensional analysis. The slope is defined as the ordinate divided by the abscissa, i. e.,

$$\frac{\text{resistance} \times \text{area}}{\text{area}} = \text{resistance}$$

In other words, the slope of the linear transformation of an alveogram has the units of "resistance." A series of different alveograms can thus be characterized by the slopes of their linear transformation, or even more simply by the value of "resistance" at an arbitrarily fixed thickness of the dough bubble wall.

The "resistance" of the dough bubble to expansion was defined earlier as the pressure divided by the bubble wall thickness. For practical purposes, a constant value for thickness may be adopted, and the alveogram pressure at that thickness may be used as the measure of "resistance." It has been found convenient to select a membrane thickness which corresponds to a distance of 2.0 cm. along the base of the alveogram; this thickness is 0.74 mm. The alveogram pressure is taken as the height in cm. along the alveogram arc. A celluloid template was constructed so that the alveograms could be read directly. The dough resistance taken as the height of the alveogram, at 2 cm. along the base, thus gives us a quantitative single figure measurement of the physical dough properties here called "resistance."

Conclusion

We have thus finally arrived at conditions necessary for a more meaningful measurement with the alveograph, namely the "resistance" of dough at a constant sample deformation. This measurement is essentially one of stress at constant strain and thus had a sound fundamental basis. In contrast, the measurements previously made with the alveograph could not be defined in terms of basic units apart from the apparatus and the conditions of testing employed. The dough resistance at constant dough thickness may now be used to extend the usefulness of the alveograph, both for practical dough testing and for studies of dough rheology. An illustration of the application of alveograph "resistance" to studies of structural relaxation in dough is given in the second paper of this series.

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CHOPIN ALVEOGRAPH STUDIES. II. STRUCTURAL RELAXATION IN DOUGH[1]

I. Hlynka and F. W. Barth

ABSTRACT

A method for studying structural relaxation in dough with the Chopin Alveograph is described. The procedure provides for a reaction time, structural activation, and rest period. The basic measurement is the dough resistance at 2 cm. along the base of the alveogram. Structural relaxation curves and data derived from them show that the alveograph gives good differentiation among doughs treated at different levels of bromate or given different reaction times. Analysis of data derived from conventional alveogram measurements (maximum pressure, area under the curve and the work function, and volume or the square root of the dough bubble at rupture), by the structural relaxation technic, showed that these measurements were of limited usefulness in fundamental dough rheology.

The physical properties of dough that has been mixed or otherwise manipulated are strikingly different from the properties of the same dough allowed to remain undisturbed for some time. For this reason the characterization of dough properties at a single point in time has limited value. A more adequate characterization of the physical properties of dough, by means of the structural relaxation curve, has been described in several publications from this laboratory (2, 3, 4).

The method of structural relaxation which was developed with the extensograph depends upon the following factors:

(a) A basic measurement of dough properties, which is neither purely empirical nor trivial, must be selected.
(b) A reaction time between mixing the dough and shaping the test piece must be allowed for reagents such as bromate to act.
(c) At the end of the reaction time dough must be structurally activated, as by rounding and shaping.
(d) Varying rest periods must be provided, between shaping and testing the sample, for the dough to relax.

A more fundamental measurement with the alveograph, namely, the "resistance" of dough to inflation referred to a constant thickness of the dough bubble, has been proposed in the first paper of this series (6). Bennett and Coppock (1) have already described a molded dough technic with the alveograph which provides for a reaction time and a

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structural activation. While their technic includes an important part of the method of structural relaxation, some of the steps are still lacking. The present paper describes the development of a complete structural relaxation method with the alveograph, based on analogous development with the extensograph, on the work of Bennett and Coppock, and on the use of the measurement of alveograph "resistance" of dough. In addition, the structural relaxation technic has been used to provide an independent confirmation of the fundamental nature of the alveograph "resistance" of dough, and a test of conventional alveograph measurements.

Materials and Methods

A commercial flour milled from high-grade Canadian spring wheats was used for this investigation. It was unbleached and contained no improvers. The absorption, as determined at a consistency of 540 farinograph units, was 61.2%, protein content 13.0%, and ash 0.44%, on a 14% moisture basis.

Doughs were prepared from 100 g. flour at an absorption of 61.2% and contained 1% salt to flour. They were mixed in air for 3 minutes in a G.R.L. mixer (5), using an open bowl under conditions to give doughs at 30°C. Twenty grams of dough were then scaled off for each test. The Petrin Extracteur supplied with the alveograph was not used, and other conditions differ from those ordinarily used with the alveograph. The object was to obtain alveograph data more comparable with the conditions employed with the extensograph and in the baking test.

Bennett and Coppock (1) described a molding technic for the structural activation of dough using an inverted funnel. Unfortunately, different doughs adhere differently to the glass funnel, and the method is therefore applicable to only a narrow range of dough properties. Figure 1 shows an improved apparatus which was modeled in some aspects on the Brabender Extensograph rounder. It consists of a small 5 × 5-cm. metal box, open at both ends, with a circular base 8.7 cm. in diameter. This box is placed on a stainless-steel plate inside a guide ring 12.8 cm. in diameter. A small nail in the center of the plate serves to tether the dough ball, and a 300-g. weighted lid, which fits loosely on top of the box, is also provided. For the structural activation, the dough sample is placed inside the box, the weighted lid is put on top, and the box is rotated by hand inside the guide ring 30 times in 12 seconds.

The next step, following structural activation of the dough to be tested, is to provide for a desired rest period without further disturb-

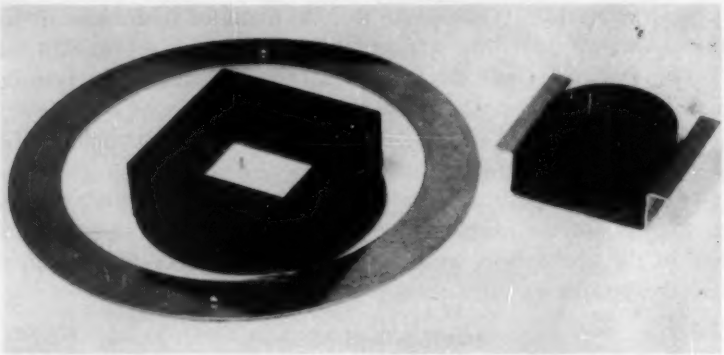

Fig. 1. Dough-rounding apparatus showing metal box inside a guide ring, and weighted lid.

ing the dough. Ideally this would be done in the dough press of the alveograph. In practice, doughs rested for extended periods in the alveograph were too relaxed and too sticky to handle. Accordingly, the dough was flattened into a disk 0.25 cm. thick by placing the dough ball on an oiled plate and pressing it for 10 seconds by means of a heavy metal block. The flattened disk, which springs back somewhat when released, was then given a rest period in a humidified cabinet. At the end of the desired rest period the sample was flattened further in the alveograph dough press and tested in the usual way.

With the foregoing procedure it was necessary to establish that no appreciable structural activation takes place during the further flattening of the sample in the alveograph dough press prior to testing. Figure 2 shows alveograms made 0, 3, 5, and 10 minutes after the dough press was turned down. All doughs were given an initial relaxation of 1 hour before they were placed in the press. The alveograms show that secondary structural activation is small and decays rapidly. On the basis of experiments of this type, it was decided to adopt 3 minutes in the dough press before blowing the bubble as adequate for the secondary activation to dissipate.

Finally, it was found that doughs given long rest periods became

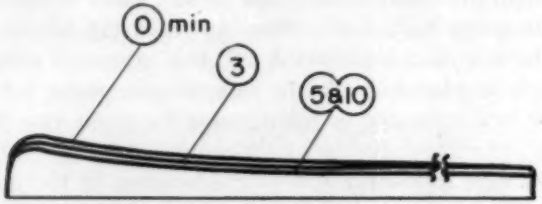

Fig. 2. Alveograms for relaxed doughs, after 0, 3, 5, and 10 minutes in dough press.

very extensible and produced bubbles larger than could be measured with the gas buret of the alveograph. Accordingly the original buret was replaced by one of 2000-cc. capacity constructed from plastic. In addition, the stop on the kymograph cylinder was removed and both ends of the chart paper were inserted under the paper clip from the right-hand side to provide a flat seam. In this manner the paper clip is covered by the paper and it is possible for the kymograph to record a second cycle if necessary.

The complete procedure thus provides for the following distinct stages: (a) reaction time, which is taken from the end of mixing to the beginning of rounding; (b) structural activation, which includes both the rounding and preliminary flattening of the dough test piece; (c) rest period, which is taken from the end of the preliminary flattening of the test piece to the actual blowing of the bubble, and includes the loading and turning down of the dough press exactly 3 minutes before the bubble is blown.

Results

The plan of the experimental work was to obtain alveogram data for doughs given reaction times of 0, 2, and 4 hours, rest periods of 5, 7, 10, 20, 30, and 45 minutes, with bromate concentrations of 0, 10, and 30 p.p.m., and flow rates of water into the alveograph air-buret of 14.9, 19.0, and 25 ml. per second. However, only representative data are summarized here. The structural relaxation data based on the alveograph are presented first. Then the traditional alveograph measurements are examined with the aid of the structural relaxation method.

Structural Relaxation Data. Figure 3 shows three representative sets of alveograms for doughs containing 10 p.p.m. potassium bromate. The sets represent three different reaction times, namely, 0, 2, and 4 hours. Each set of alveograms shows that as the rest period increases from 5 to 45 minutes the alveograms become lower and longer. The influence of the action of potassium bromate is shown by comparisons of the three sets of alveograms, since these represent differences only in reaction times. The increase in height and the decrease in the length of the alveograms with increasing reaction time illustrate the bromate effect. The picture is entirely analogous to that obtained with the extensograph.

Linear transformations of these alveograms over a length approximately corresponding to the shortest alveogram are shown as insets in Fig. 3. The area of the dough bubble is plotted against the product of the area and the dough resistance as described in the first paper (6).

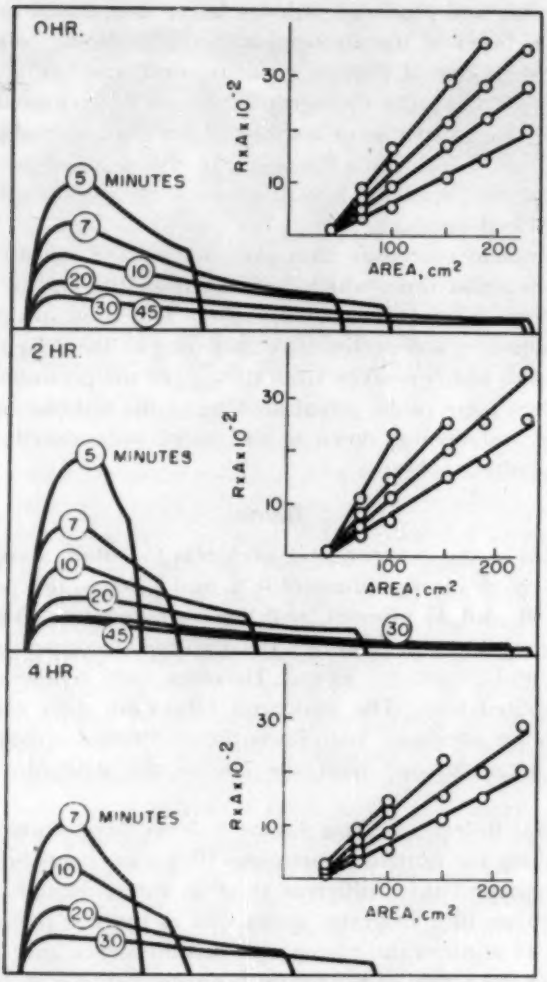

Fig. 3. Alveograms showing effect of rest period on doughs containing 10 p.p.m. bromate and given reaction times of 0, 2, and 4 hours. Inset, linear transformations of these alveograms.

These and many other sets of data that were examined establish clearly that alveograms obtained under a wide variety of conditions are mathematically related and that the alveogram resistance at constant sample deformation may be used as a general physical measurement.

Figure 4, left, shows representative structural relaxation curves obtained from alveograms. Doughs were given reaction times of 0, 2, and 4 hours, and contained 0, 10, and 30 p.p.m. potassium bromate. Alveogram resistance was measured directly as alveogram height along

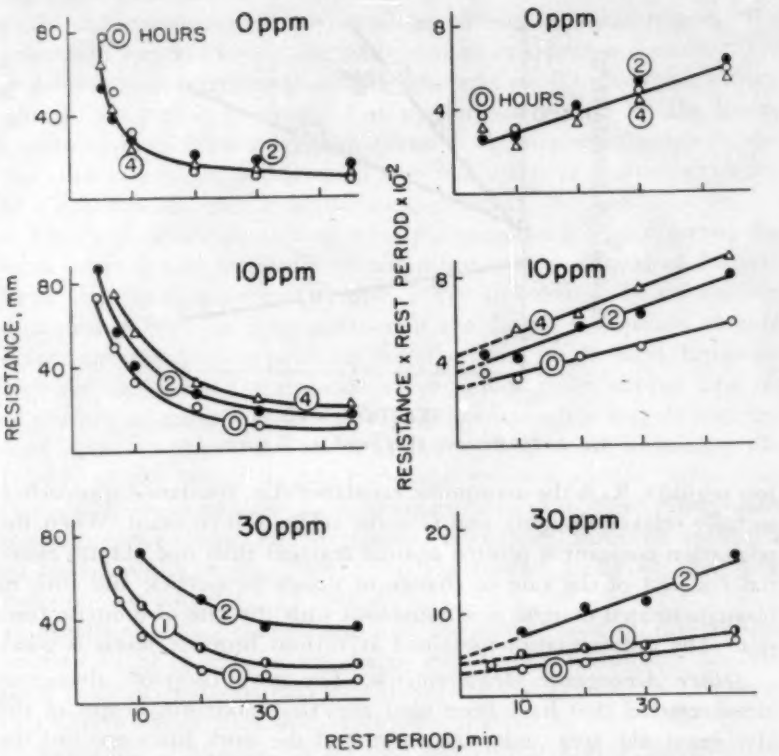

Fig. 4. Structural relaxation curves based on alveogram data, and linear transformations of these.

the arc, at a sample extension corresponding to a length of 2 cm. along the base of the alveogram, and thus at a standard bubble-wall thickness of 0.74 (cf. reference 6).

To test the structural relaxation curves, linear transformations (shown at the right of Fig. 4) were obtained by plotting the rest period against the product of the rest period and dough resistance (2). The linearity of these plots indicates that structural relaxation curves obtained from alveogram data are entirely satisfactory; the linearity also provides independent confirmation that alveogram resistance is a basic measurement of dough properties.

Figure 5 shows that a satisfactory degree of differentiation is obtained at various bromate levels in reaction rate plots. Here, relaxation constants C were calculated from structural relaxation data, assuming that the relaxation curves approximate a hyperbola (2). Mathematically, $(R - R_A)\,t - C = 0$, where R is the alveogram resistance at

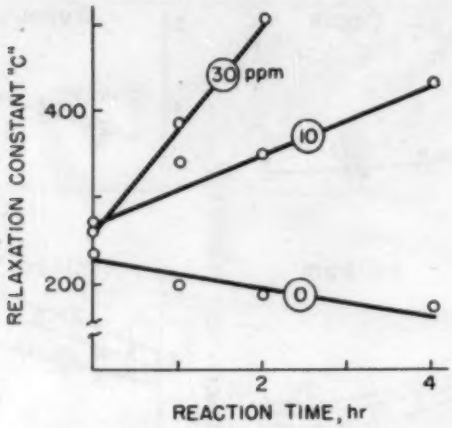

Fig. 5.-Reaction rate plots based on alveogram data.

rest period t, R_A is the asymptotic resistance (i.e., resistance approached in fully relaxed doughs), and C is the relaxation constant. When the relaxation constant is plotted against reaction time one obtains essentially a plot of the rate of change of dough properties, and this, in bromate-treated doughs, is synonymous with the rate of bromate reaction. The differentiation obtained at various bromate levels is good.

Other Alveogram Measurements. The most common alveogram measurements that have been used are: the maximum height of the alveogram, the area under the curve and the work function, and the square root of the volume of the dough bubble as a measure of extensibility. These measurements have been examined in relation to the structural relaxation method and the results are summarized below.

The main shortcoming of the measurement of the alveogram maximum lies in the fact that the maximum may refer to one thickness of the dough bubble in one alveogram and to a different thickness in another alveogram. The pressure at the maximum is thus a function not only of dough properties but also of dough thickness.

The variation in thickness of the dough bubble at alveogram maximum has been examined for a wide variety of conditions available in this study. Representative results may be cited for untreated dough given a zero reaction time. The thickness of the dough-bubble wall varied from 0.68 mm. for a dough rested 5 minutes to 1.04 mm. at 45 minutes. These results may be considered typical. Unless the doughs tested are very similar in properties, the error due to the variation of the dough thickness may be considerable. It may, of course, be very simply obviated by using the resistance of dough at constant sample

deformation as already described.

In the assessment of the remaining alveogram measurements the procedure was to plot (1) the measurement in question against alveogram resistance at constant sample deformation, (2) the measurement against rest period to obtain a relaxation curve, and (3) the linear transformation of the relaxation curve. A definite relationship in the first and a linearity in the third case was taken as positive evidence of a fundamental nature of the measurement.

The areas under alveograms were measured with a planimeter, the work functions were calculated according to the manual of instructions, and the data were examined. There appeared to be no obvious relationship between the resistance of the dough at constant sample deformation and the area under the alveogram or the work function. Nor did the "linear transformation" yield more information. The results obtained in this work with alveogram resistance suggest that the work function should be taken as the work done in increasing the volume of the dough bubble from zero to some specific value, corresponding for example to 2 cm. along the alveogram base, rather than over the entire range. The alveogram "resistance," however, appeared to be a simpler measurement, and no further study of the work function was made.

Finally, data on the volume and the square root of the volume at the time of rupture of the dough bubble were examined. Because these measurements increase with increasing rest period, their reciprocals were used for structural relaxation analysis.

Structural relaxation curves and the corresponding linear transformations were obtained from each measurement. The results showed a general relationship similar to those obtained using alveogram "resistance." There was, however, a much larger experimental error in the reproducibility of individual results. The curves based on the square root of the volume appeared to be somewhat better than those based on the measurement of volume. While these observations appear to be interesting, they were not considered to be sufficiently significant at this stage to warrant presentation in greater detail.

Discussion

The investigations described in this and the previous paper were not intended to give new information about dough properties but rather to provide a new and more meaningful method of using a well-established dough-testing instrument. It has been shown that the alveograph can be adapted for structural relaxation studies of dough. The technic of handling the dough is slightly more complex than

with the extensograph. However, only 20 g. of dough are required for a single alveogram, and this feature is of some interest to those who wish to test flour quality when only small amounts of flour are available.

The structural relaxation method has also provided independent means of assessing the value of alveogram measurements that have been used for many years. The pressure at the alveogram maximum has been shown to be a function not only of the physical properties of dough but also of the thickness of the dough-bubble wall. The results based on the area under the alveogram and the work function were disappointing. The results based on the measurement of the volume of the dough bubble at rupture or the square root of this volume could, broadly speaking, be used in the same way as alveogram resistance, but they lacked a satisfactory degree of precision. While the traditional alveogram measurements may have some value as empirical indexes of dough properties, their use for basic studies of dough rheology appears to be limited.

Mechanically, the alveograph is a distinctly different instrument from the extensograph. What information it can yield that cannot be obtained with other instruments is an interesting question for future research.

The present investigation was undertaken to provide some of the background of basic knowledge required to make such research possible.

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HYSTERESIS IN THE HYGROSCOPIC EQUILIBRIA OF ROUGH RICE AT 25°C.[1]

MICHAEL H. BREESE[2]

ABSTRACT

Hysteresis in the hygroscopic equilibria of rough rice at 25°C. was measured at relative humidities from 10 to 90%. A difference greater than 1% between the adsorption and desorption equilibrium moisture contents was maintained over the range of relative humidities from 20 to 80%, but exceeded 1.5% only at relative humidities from 50 to 70%. Rough rice at moisture contents between 12.6 and 14.1% may be in equilibrium with a relative humidity of 75%.

The moisture contents at which rough rice (paddy) will store satisfactorily under the normal conditions of the wetter tropics lie between fairly close limits. Del Prado and Christensen (4) have shown that at temperatures between 17° and 24°C. mold growth increases with increasing moisture content above 15%, and the viability of the seed decreases. The role of fungi in causing heating and spoilage in grain with moisture contents in equilibrium with relative humidities of 75% and above has been demonstrated by Milner, Christensen, and Geddes (9) and by many other workers. A definite upper limit of 15% (wet-weight basis) may therefore be set on the moisture content of rough rice for storage. For safe storage in ordinary bins of wood or steel, even over a short period, Coonrod (3) has advocated a maximum moisture content of 13.0–13.5%. A minimum limit just above this figure has, however, been suggested by Stahel (10). Working in Surinam with rough rice of the Vary Lava, Baok, and Skrivimankoti groups, he showed that if water adsorption took place in rice having a moisture content below 14%, cracks, misnamed "suncracks," developed in the kernels. These cracks are responsible for most of the breakage in milling, and Stahel therefore recommended that rough rice to be stored should not be dried below 14% moisture content unless kept in a container that would safeguard it against water uptake.

The hygroscopic equilibrium of rough rice at 25°C. was determined by Coleman and Fellows (2), and of rough rice and rice fractions by Karon and Adams (8). Neither of these studies, however, included a demonstration of hysteresis which has been shown to influence the equilibrium moisture content of wheat and of brown and whole-grain edible forms of rice. Babbitt (1) found that in wheat, hysteresis caused

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a moisture content difference of over 4% at relative humidities from 20 to 40%, and that the effect only diminished markedly above 60% r.h. Houston (6) showed that when Caloro brown rice was brought into equilibrium at 40–65% r.h. by desorption, the moisture content was 0.7% to 1.0% higher than that attained by adsorption. Hysteresis effects in the sorption of moisture were shown by Houston and Kester (7) to be greater in parboiled and quick-cooking rices than in raw white and brown rices. An indication of the hysteresis effect in rough rice may be obtained from the figures given by Karon and Adams for the equilibrium moisture content at 70 and 80% relative humidity. Where equilibrium has been attained through desorption, the moisture content at a given relative humidity is significantly higher.

To determine the extent of hysteresis over a range of relative humidities from 10 to 90%, a study was made of the equilibrium moisture contents of rough rice attained by adsorption and desorption.

Materials

Short-grain Joya, a Trinidad-grown rice variety, probably of Indian

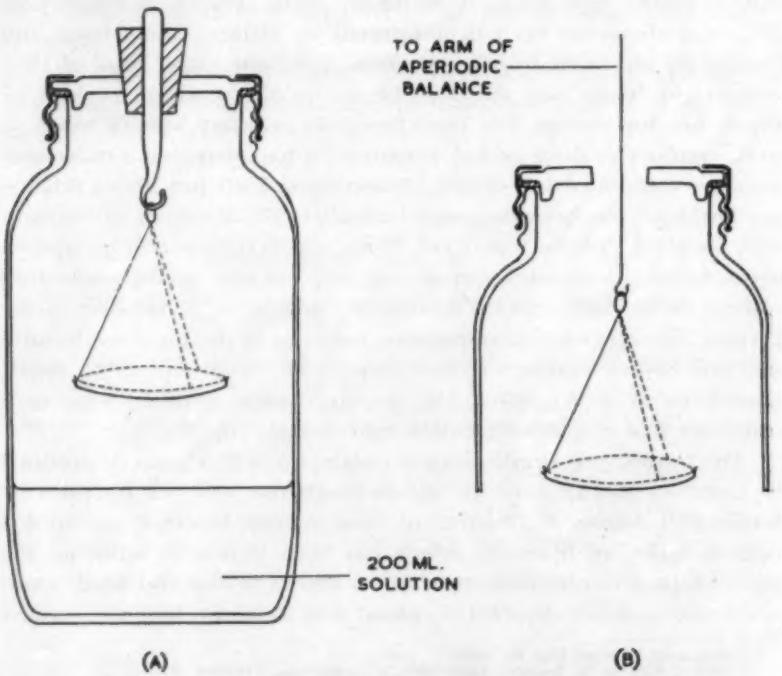

Fig. 1. A, cross section of conditioning apparatus. B, method of weighing.

derivation, was used in the experiments. The rough rice was from the 1933 (November) harvest and had been field-dried to a moisture content of 13.1%. The sample was divided into two parts, the first of which was dried intermittently in a ventilated oven to a determined moisture content of 5.8%, and then in a vacuum oven until a moisture content of 3.3%, calculated from the further loss of weight incurred, was reached. Drying temperatures never exceeded 70°C. The second portion was conditioned for more than 4 months to a relative humidity of 90%, and its final moisture content was determined as 16.1%.

Methods

The apparatus used (Fig. 1, A) was essentially that of Gane (5), except that the glass lids of the 2-lb. Kilner jars were drilled to take an 18-mm. diameter rubber bung carrying a short piece of glass rod drawn into a hook at the lower end. A shallow aluminum dish holding a weighed quantity (about 2 g.) of grain was suspended from the hook. By means of an accurately counterpoised piece of light wire, the dishes could be suspended from the arm of an aperiodic balance and weighed within the jars to 0.1 mg. (Fig. 1, B). Weighings were made daily during the early stages of equilibration, then at increasing intervals. Equilibration was continued for 56 days. From the gain or loss in weight incurred by the grain, the moisture content at equilibrium or at any point during equilibration could be calculated. Relative humidities from 10 to 30% inclusive were controlled by sulfuric acid solutions, and from 40 to 90% by solutions of calcium chloride; 200 ml. of solution, made up in both cases to the concentrations given by Stokes and Robinson (11) were used in each jar.

Weighings were made in a room in which the temperature was controlled between 23° and 24°C. Between weighings the jars were kept in an incubator located in the same room, at 25° ± 0.2°C.

All moisture content determinations were made by the "standard" method for the United Kingdom and Colonial Territories. This is a two-stage method involving preconditioning for 18–40 hours, grinding so that all of the kernels pass through a 0.84-mm. (No. 20 American) screen, and heating in an electric oven with mechanical convection for 1 hour at 130°C. In all important respects this method is identical with the two-stage, air-oven method specified in the Handbook of the Official Grain Standards of the United States (12).

A hand-operated "Spong" coffee mill, as permitted in the "standard" specifications, was used to grind samples. It was found that with small amounts (about 2 g.) of rough rice, a single passage through this mill, at settings which did not generate too much heat, did not give

the requisite degree of fineness. In determining the equilibrium moisture contents attained by adsorption, all samples were ground twice. The results indicated, however, that this procedure tended to dry the majority of samples. In subsequent determinations, small samples were ground only once. This proved to be sufficient, and acceptable moisture determinations were for the most part within 0.2% moisture of the values calculated by weight changes. All moisture contents are given on a wet-weight basis.

Results

The equilibration of rough rice by adsorption and desorption to relative humidities of 10–90% is shown in Fig. 2 and Fig. 3 respectively. Equilibration by adsorption is extremely rapid at relative humidities above 50%, more than 96% of the eventual water uptake taking place during the first 4 days. Figure 3 shows that in desorption the exchange of water is not quite so rapid and that the equilibrium moisture contents are higher. The extent of this hysteresis is shown in Table I and Fig. 4. The difference between adsorption and desorption equilibrium moisture contents is not large and exceeds 1.5%

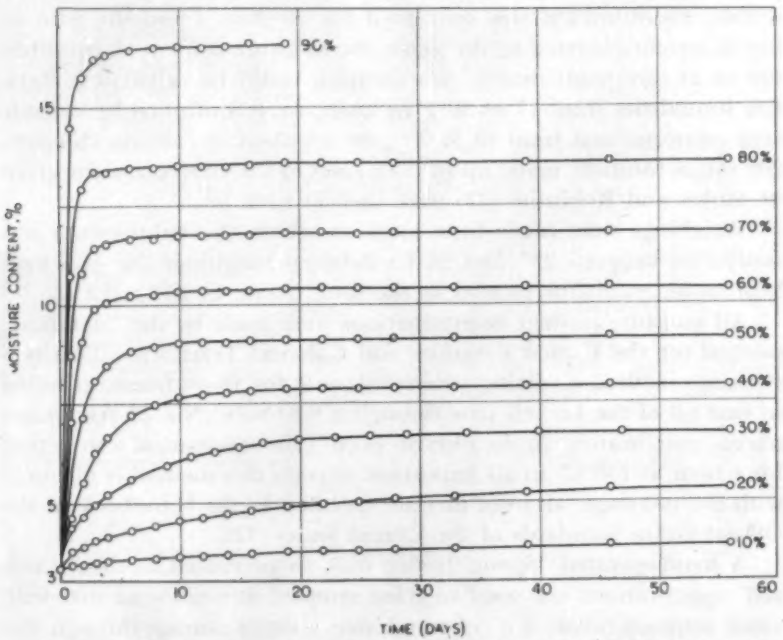

Fig. 2. Equilibration of rough rice by adsorption to relative humidities from 10 to 90% at 25°C.

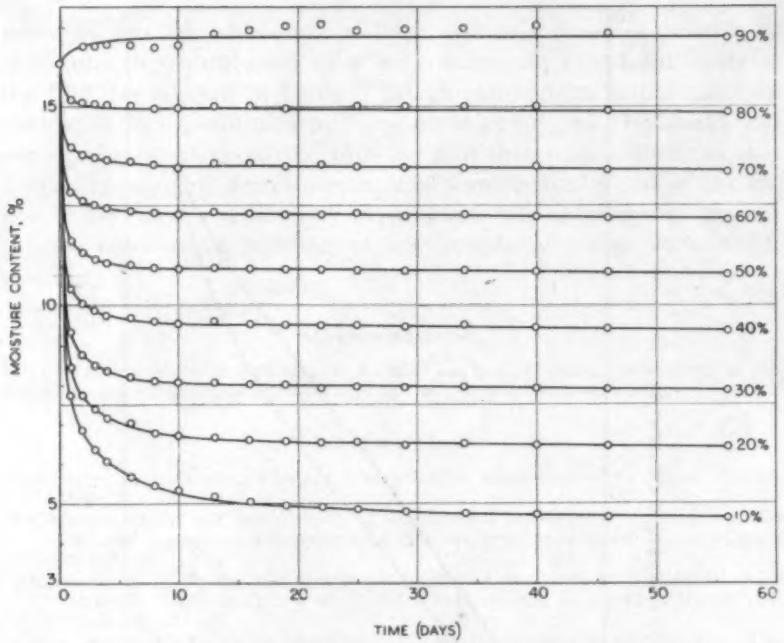

Fig. 3. Equilibration of rough rice by desorption to relative humidities from 10 to 90% at 25°C.

TABLE I

EQUILIBRIUM MOISTURE CONTENTS OF ROUGH RICE ATTAINED BY ADSORPTION AND DESORPTION AT DIFFERENT RELATIVE HUMIDITIES AT 25°C.

RELATIVE HUMIDITY	ADSORPTION Initial Moisture Content 3.3% Calculated Value	DESORPTION Initial Moisture Content 16.1% Calculated Value	DIFFERENCE BETWEEN CALCULATED VALUES
%	%	%	%
10	3.9	4.6	0.7
20	5.3	6.5	1.2
30	6.8	7.9	1.1
40	7.9	9.4	1.5
50	9.2	10.8	1.6
60	10.4	12.2	1.8
70	11.8	13.4	1.6
80	13.6	14.8	1.2
90	16.6	16.7	0.1

only from 50 to 70% r.h. A difference of over 1% is, however, maintained throughout the range 20–80% r.h. Rough rice at moisture con-

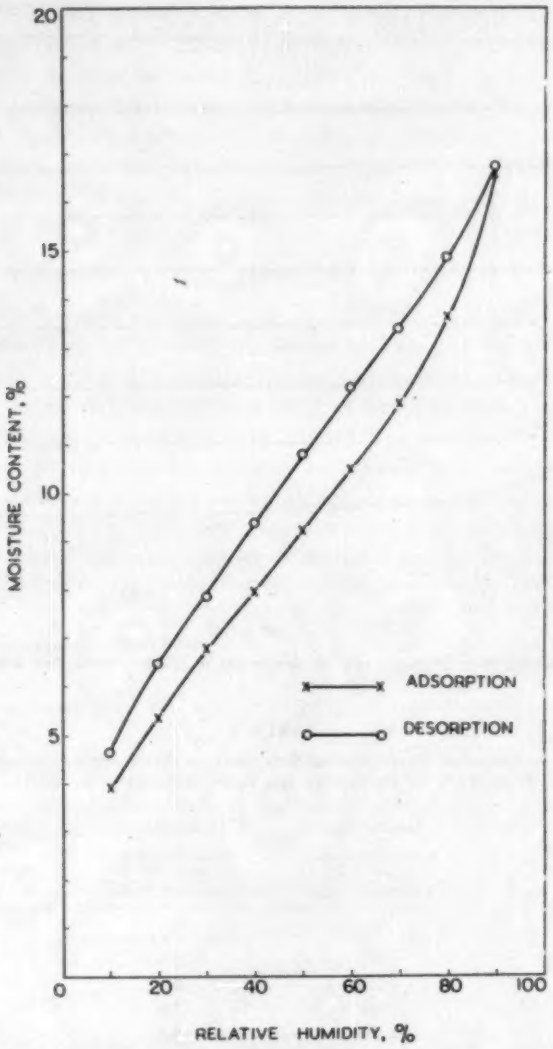

Fig. 4. Equilibrium moisture contents of rough rice attained by adsorption and desorption at relative humidities from 10 to 90% at 25°C.

tents of 12.6% to 14.1% may be in equilibrium with a relative humidity of 75%. The calculated hygroscopic equilibria for desorption show a very close agreement with those obtained by Karon and Adams (8) for field-dried rough rice.

Molds developed rapidly after 19 days in the dry rough rice ex-

posed to a relative humidity of 90%, and as it was not possible to determine the equilibrium moisture content, the calculated figure at the 19th day is given in Table I. Rough rice with an initial moisture content of 16.1% still took up some water at 90% r.h., but molds did not develop until about the 46th day and then only slightly, so that a moisture content determination was possible at the end of the exposure period. No satisfactory explanation can be offered at present for the more rapid molding of one sample of rough rice at this humidity.

Acknowledgment

The author is grateful to Mrs. T. E. Wise for her assistance, particularly in the determination of moisture contents and in the preparation of solutions.

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THE DIFFUSION OF CARBON DIOXIDE THROUGH FERMENTING BREAD SPONGES [1]

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ABSTRACT

Radioactive carbon dioxide was used to test the permeability of fermenting sponges to carbon dioxide. Results showed that the carbon dioxide retained above a fermenting dough in cabinet fermentation is easily capable of diffusing back into the sponge even against the developing internal gas pressure of the mass. The data support the free diffusion theory suggested by Sullivan and Richards (6).

The fermentation of bread sponges in a restricted space is referred to as cabinet fermentation and has been the subject of several critical investigations. Conclusions on the merit of this procedure, however, have been conflicting and the subject remains a matter of controversy.

Schoonover, Freilich, and Redfern (5) studied open trough and cabinet fermentation under varying conditions of doughroom temperature and humidity, sponge temperature, and yeast percentage. They reported that the confining of carbon dioxide above a fermenting sponge gave a dough of improved characteristics only when the temperature of the fermentation room was definitely cooler than that of the starting temperature of the sponge. When, however, doughroom temperature equaled or was higher than that at which the sponge was set, no advantages were noted. These authors concluded that cabinet fermentation has no advantage over the open trough type in shops having accurate temperature and humidity control. Garnatz, Hodler, and Rohrbaugh (2) in their evaluation of cabinet and open trough fermentations found no significant differences between the methods either in the physical properties of the doughs or in the bread produced. They agreed with Schoonover and coworkers that the only advantage of the cabinet is in minimizing fluctuations in doughroom temperature and in automatically providing sufficient humidity to prevent crusting of the sponges.

On the other hand, Johnson (3), in a series of laboratory-scale baking tests, found that an atmosphere composed of 15.5 to 25.0% carbon dioxide, 14.0 to 15.5% oxygen, and 61.0 to 69.0% nitrogen and a 3-hour sponge time produced better bread than the control dough fermented in normal air. Sullivan and Richards (6), in a comparison

[1] Manuscript received April 14, 1953.
[2] College of St. Catherine, St. Paul, Minnesota.
[3] Russell-Miller Milling Company, Research Laboratory, Minneapolis, Minnesota.

of open trough and cabinet fermentation of sponges, found the cabinet method to be superior. They showed that a high concentration of carbon dioxide above the sponge after the "break" resulted in the production of drier, mellower doughs that machined more smoothly and gave bread having a finer grain and softer texture. In contrast, an atmosphere of oxygen or nitrogen above the sponge gave doughs with poor handling characteristics resulting in inferior bread. To account for the effect of these gases on the development of the bread doughs, Sullivan and Richards advanced the "free diffusion" theory. They suggested that the sponge is permeable to the gases above it and that the superiority of cabinet fermentation is due to the fact that carbon dioxide released after the sponge breaks is retained inside the cabinet and thus had adequate opportunity to diffuse back into the sponge even against the high internal pressure of the continually evolved carbon dioxide.

To test the validity of this theory, the present investigation was begun. In order that the movement of carbon dioxide through the sponge might be followed without disturbing the normal course of the fermentation and without distorting the sponge, the phenomenon was investigated using radioactive carbon dioxide ($C^{14}O_2$) as a tracer gas.

Materials and Methods

In these experiments the sponges were made up according to the formula previously reported (6), but on a laboratory scale. The formulation of the sponge was as follows:

	Weight g
Short patent flour	240.0
Water	144.0
Yeast	8.0
Lard	12.0
Yeast food – Arkady	0.8

Immediately after mixing, the sponges were placed in a can 7½ in. × 6⅛ in. and allowed to stand at room temperature (approximately 80°F.) until the break was observed. This customarily took place approximately 2 hours after mixing. The can serving as the "cabinet" in these experiments was provided with a removable bottom and was open at the top. The atmosphere of carbon dioxide above the sponge was confined by means of a large watch-glass which was placed over the top of the can. The sponge at the "break" filled the can to a point 4–5 in. from the top.

Soon after the breaking of the sponge was noted, the carbon dioxide

obtained by acidifying 1 g. of barium carbonate containing an appropriate amount of barium – C^{14} – carbonate was introduced into the cabinet immediately above the surface of the dough. The system was then allowed to stand with the radioactive carbon dioxide confined above the sponge. Various intervals of exposure, from 30 minutes to 2 hours, were used. At the end of the contact period, the can was carefully opened and the space above the sponge gently vacuum cleaned to remove carbon dioxide. The can was then placed in an ice chest, covered with a loosely fitting metal lid, and packed with dry ice, care being taken to disturb the dough as little as possible during these operations. After 0.75 to 1.25 hour, the container was removed from the dry ice and the sponge was found to be frozen to a solid, brittle mass. The bottom cover was removed from the can and a plug was cut out of the dough from bottom to top using a No. 15 cork borer. In each experiment, two plugs were cut from different parts of the sponge, one from the center and one from the side near the wall of the container. The plug thus removed from the sponge was carefully extruded from the cork borer and cut into three sections representing the top, center, and bottom areas of the sponge. Each of these sections was introduced into a flask containing 100 ml. of 0.5 *M* sodium hydroxide. The flasks were then stoppered and the samples allowed to stand from 48 hours to 1 week, with occasional shaking, to allow disintegration of the dough sections.

At the end of the disintegration period, the contents of each flask were emptied into a 200-ml. reaction vessel which was attached to a standard carbon dioxide absorption train such as that described by Kolthoff and Sandell (4) but provided with a carbon dioxide absorber of aqueous sodium hydroxide rather than one of ascarite. The mixture in the reaction flask was then strongly acidified and heated to boiling, and the evolved carbon dioxide was collected in the aqueous alkali of the absorber. The double collection of carbon dioxide was necessary because, in a single collection, it was found to be impossible to free the original alkali solution from gluten and gelatinous materials of the dough by any type of filtration attempted or by centrifuging. The gummy materials contaminated all barium carbonate plates precipitated from the original alkaline solution and these contaminated plates could not be dried without cracking badly.

The carbon dioxide collected in the absorption bottle of the gas train was then precipitated as barium carbonate using the barium chloride-ammonium chloride technic described by Calvin (1). The barium carbonate plates thus produced were counted in a flow-gas counter. Results of these experiments are presented in Table I.

TABLE I

DIFFUSION OF CARBON DIOXIDE THROUGH FERMENTING DOUGH

Run No.	Activity Added	Exposure Time	Area of Sponge Sampled	Weight of Sample	Counts per Minute		
					Background	Corrected	Per Gram
	mc	*hours*		*g*			
1	0.139	2	Center				
			Top	...	36	147	...
			Center	...	36	495	...
			Bottom	...	36	175	...
			Side				
			Top	...	36	187	...
			Center	...	36	169	...
			Bottom	...	36	177	...
2	0.377	2	Center				
			Top	1.54	38	807	524
			Center	1.83	38	1167	638
			Bottom	1.76	38	1634	928
			Side				
			Top	1.71	42	475	278
			Center	2.89	38	701	243
			Bottom	2.86	38	998	349
3	0.524	0.5	Center				
			Top	...	36	1593	...
			Center	...	32	761	...
			Bottom	...	34	1054	...
			Side				
			Top	...	33	947	...
			Center	...	32	581	...
			Bottom	...	34	1193	...

Results

The data of Table I clearly indicate that the radioactive carbon dioxide, although introduced only into the space above the sponge, diffused into every section of the fermenting mass. The distribution of radioactivity throughout the dough appears to be entirely fortuitous and is that to be expected from the principles of free diffusion and the vesicular nature of the fermenting sponge. These results give support to the free diffusion theory of Sullivan and Richards.

As expected, increasing the activity of the gas introduced into the cabinet was accomplished by a definite increase in the radioactive character of all samples (compare the data in run 1 with those in run 2). That the rate of diffusion of the gas through the sponge is comparatively rapid is shown by run 3 which displays radioactivity throughout the dough mass after only 0.5 hour's exposure to the tracer carbon dioxide.

It should be emphasized that these data are qualitative. Quantitative studies necessary to describe the rate of this process would have to provide for reporting counts per minute on the basis of the gas volume included in each section of the sponge. The measurement of gas volume in a fermenting sponge is exceedingly difficult and, in any case, such rate studies were beyond the scope of this investigation.

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A COMPARISON OF THE EFFECTS OF BROMATE AND CALCIUM STEARYL-2 LACTYLATE ON BREAD QUALITY[1]

J. B. THOMPSON[2] AND B. D. BUDDEMEYER[3]

ABSTRACT

Calcium stearyl-2 lactylate caused an improvement in bread quality similar in extent and kind to that brought about by potassium bromate. The effects of the two improvers were additive but were probably due to different modifications of the dough.

The beneficial effects resulting from the use of extremely minute quantities of various oxidizing agents in the flour employed in bread production have been recognized for some time. Agents such as the bromates, iodates, and various peroxides at present are generally employed by the baking industry; and there is no doubt that with most flours their use in appropriate amounts results in bread of improved symmetry, volume, grain, and acceptability.

Thompson and Buddemeyer (4) recently reported the effects of calcium stearyl-2 lactylate on flour properties and bread quality. The pronounced beneficial action of this material on loaf volume, grain, symmetry, texture, and similar quality factors at the optimum level greatly resembled the action of oxidizing agents qualitatively though not quantitatively. Although the calcium stearyl-2 lactylate is not an oxidizing agent, its action possibly involves the same active groups as those upon which bromate and similar agents are postulated to have their effects.

The purpose of this investigation was to compare the effects of potassium bromate and calcium stearyl-2 lactylate at various levels, both singly and in combination, on the dough and baking qualities of a sensitive and responsive flour and thereby to ascertain the magnitude of their individual effects.

Materials and Methods

The flour employed in this investigation was the same type of 95% baker's patent (11.4% protein and 0.45% ash on a 14.0% moisture basis) used in the previous study (4). It was known to respond well to calcium stearyl-2 lactylate but required only 0.5% yeast food for optimum bread quality. The flour was obtained in an

[1] Manuscript received June 9, 1955. Contribution from the C. J. Patterson Co., Kansas City, Missouri.
[2] Present address; Pillsbury Mills, Inc., Springfield, Illinois.
[3] Present address; Midwest Research Institute, Kansas City, Missouri.

unoxidized and unbleached state to increase its requirements for, and maximum response to, potassium bromate.

A farinograph curve made on the flour indicated an optimum absorption of 63.0%, a dough development time of 5 minutes, and a decrease in consistency of 40 Brabender units when mixed for 5 minutes beyond maximum dough consistency. These data were used to establish optimum conditions for the baking tests.

A bromate-free yeast food was formulated in the laboratory in lieu of the standard yeast food employed (2). This special yeast food salt mixture was composed of 25% calcium sulfate, 10% ammonium chloride, 25% sodium chloride and 40% wheat starch. It was prepared by weighing the ingredients into a closed container and vigorously agitating until a homogeneous mixture was obtained.

The potassium bromate was incorporated in the form of a dilute aqueous solution, each milliliter equivalent to 0.0053 g. potassium bromate, a quantity of bromate equal to the amount normally contained in yeast food employed at a 0.25% (flour basis) level in a 700-g. flour sponge and dough formula. This is equivalent to 0.757 mg. bromate per 100 g. flour.

The calcium stearyl-2 lactylate was a commercial product,[4] the chemical and physical properties of which have been described previously by Thompson and Buddemeyer (4). This material was used at various levels up to 2.00% of the flour and was introduced as a dry powder at the dough stage.

Baking. The baking procedure used for the preparation of test bread was of the sponge and dough type utilizing the following commercial formula:

Ingredients	*Weight*
	g.
Flour	700.0
Water	441.0
Yeast	16.0
Yeast food salt mixture	3.5
Potassium bromate	Variable
Nonfat milk solids	21.0
Sugar	56.0
Lard	14.0
Salt	16.0
Calcium stearyl-2 lactylate (VERV-Ca)	Variable

Seventy percent sponges were prepared using 279 g. of the total water with addition of the yeast, yeast food salts, and bromate solution, if employed. In those sponges containing the aqueous bromate solution, compensation in sponge absorption was made for this additional water. The sponges were mixed 1 minute at low speed followed by 1

[4] VERV-Ca, product of the C. J. Patterson Company, Kansas City, Mo.

minute at second speed in a jacketed McDuffee bowl on a Hobart A-200 mixer. The sponges came from the mixer at 80°F. (26.7°C.) and were fermented for 4.5 hours at 84°F. (28.9°C.) and 85% relative humidity.

The doughs were prepared by placing the remainder of the dry ingredients, including the calcium stearyl-2 lactylate, when employed, and the water in the mixing bowl and starting the mixer at low speed. Mixing was continued for 3 minutes while the fermented sponge was being added in four approximately equal parts.

The doughs were mixed for 4 minutes at second speed and were brought from the jacketed mixer at 80°F. (26.7°C.). They were given 45 minutes' floor time in the fermentation cabinet and divided into two 539-g. dough pieces. These dough pieces were made up through rounder, overhead, and molder in the commercial manner and proofed to equal volume at 106°F. (41.1°C.) and 90% R.H. The bread was baked at 425°F. (218.3°C.) for 23 minutes.

Calcium stearyl-2 lactylate was tested singly at levels in the range 0.25 to 2.00%. Potassium bromate was evaluated at test levels from 0.0053 g. to 0.0630 g., amounts equivalent to the quantity of potassium bromate contained in 0.25 to 3.0% of Arkady-type yeast food, based on the flour weight. In addition, the two adjuncts were included in the bread formulation in varying amounts. The exact levels and combinations are given in the table of results.

Tests on Bread. The finished loaves of bread were permitted to cool 1 hour, and their volumes were determined by the rapeseed displacement procedure. The loaves were packaged in airtight polyethylene bags and stored at room temperature on an open bread rack at spaced intervals of 2 inches to permit free circulation of air, for 18 hours prior to examination.

The scoring of the loaves was performed by three experienced individuals using the system of the American Institute of Baking. The scores take into account such external factors as volume, color, symmetry, and break and shred; these external characteristics account for 30% of the theoretical 100 score. The internal characteristics—grain, crumb color, texture, and edibility factors—constitute the remainder.

Crumb compressibility was determined by the method of Bradley and Thompson (1) with certain variations. The instrument employed was a precision penetrometer of the type specified by the American Society of Testing Materials in which the compressing plunger was a cylinder 3 cm. in diameter and loaded so that the whole plunger weighed 265 g.

The compressibility measurements were made on three slices from

each of six loaves. Each loaf was sliced in 2-in. sections using a sharp knife and a miter box. The penetrometer plunger was brought to the slice surfaces and released for 10 seconds; readings in 0.1 mm. of penetration were read from the dial micrometer of the instrument. The average of eighteen such readings constitutes each compressibility value.

These compressibility values made after 18 hours reflect such quality factors as loaf volume, grain, texture, and mastication character, which were also evaluated subjectively.

Results and Discussion

The data obtained from these investigations are presented in Table I.

The bromate series revealed that potassium bromate materially improved the bread prepared from an untreated flour, with regard to volume, compressibility, and quality. The inclusion of potassium bromate caused an increase in loaf volume, but the maximum was approached at 0.757 mg. per 100 g. of flour, or the lowest level employed. The bread quality score increased gradually from 69 for the control to a maximum of 83 obtained with 6.057 mg. of bromate per 100 g. flour in the formula. This bromate dosage corresponds to the oxidizing equivalent of 2% yeast food and indicates that about three-quarters of the oxidation requirements of a flour of the type employed are met in the mill through bleaching and maturing treatments, since flour so treated required only the bromate equivalent to 0.5% yeast food. Bromate increased compressibility considerably and in a manner paralleling the increase in quality.

The inclusion of calcium stearyl-2 lactylate alone in the formula caused a gradual increase in volume, quality, and compressibility of the test loaves with the level used. A maximum in all three evaluations was obtained with about 1.5 g. per 100 g. of flour. The maximum compressibility was greater than was obtained in the bromate series; the bread quality was substantially improved over the control, but the maximum was slightly lower than in the bromate series. The increase in volume with additions of calcium stearyl-2 lactylate was more constant than that obtained with bromate and resulted in larger maximum size.

Further baking tests were made, using combinations of the two baking adjuncts; in each, the adjunct selected to be held constant was used at a level somewhat below the optimum as revealed for the materials alone. This was to allow for observable improvements.

The addition of increments of lactylate to a dough containing

TABLE I

INFLUENCE OF POTASSIUM BROMATE AND CALCIUM STEARYL LACTYLATE ON BREAD CHARACTERISTICS

ADJUNCTS TO FLOUR		LOAF VOLUME	CRUMB COMPRESSIBILITY	QUALITY SCORE [a]
Potassium Bromate	Calcium Stearyl Lactylate			
mg/100g	*g/100g*	*cc.*	*0.1 mm*	
Control		2800	127	69
0.757		3025	159	71
1.514		3025	169	73
2.271		3000	175	75
3.029		2950	173	79
4.543		3075	182	81
6.057		3100	179	83
9.086		3000	184	82
	0.25	3075	180	70
	0.50	3100	196	71
	0.75	3100	197	72
	1.00	3125	203	75
	1.50	3200	222	80
	2.00	3200	218	79
1.514		3050	173	76
1.514	0.25	3225	182	79
1.514	0.50	3250	224	84
1.514	0.75	3300	218	84
1.514	1.00	3350	215	83
1.514	1.50	3275	205	84
1.514	2.00	3250	206	83
	0.50	3050	191	71
0.757	0.50	3225	197	79
1.514	0.50	3250	228	83
2.271	0.50	3250	223	84
3.029	0.50	3300	230	85
4.543	0.50	3375	249	87
6.057	0.50	3250	227	83
9.086	0.50	3175	207	84

[a] Relative score using system of the American Institute of Baking.

1.514 mg. of bromate per 100 g. flour, which is less than the usual amount of oxidizing improver which a flour receives at the mill, resulted in a significant improvement in quality up to a level of 0.50 g. per 100 g. of flour. The effects of the two agents on crumb compressibility and volume were similar.

The inclusion of potassium bromate in the sponges used to prepare doughs containing 0.50 g. of calcium stearyl-2 lactylate resulted in increased quality, compressibility, and volume to a level of 4.543 mg. of bromate per 100 g. of flour. These were the best breads obtained in the course of the investigation.

The similarity of action of potassium bromate alone and calcium stearyl-2 lactylate alone was very pronounced. The subjective examination of the test loaves prepared from the untreated flour containing either bromate or lactylate alone did not reveal any distinctive characteristics which would permit certain identification of the adjunct employed.

These experiments demonstrate conclusively that the improving effects of bromate and the lactylate are additive, as indicated by the criterion of effectiveness employed: this is particularly notable in the series containing a constant level of bromate and variable additions of lactylate. Not only are these effects additive, but the materials apparently are somewhat synergistic: this action is evident at the lower levels of combination.

A further indication of the difference in action is provided by the amounts of bromate and lactylate required for comparable effectiveness. Very small quantities of bromate sufficed, whereas a quantity of the lactylate about 200 times that weight was required to cause similar improvements in the bread character.

Sullivan *et al.* (3), in a study of the effects of bromate on the physical properties of flour dough, have indicated that this material does not produce any discernible changes in these properties until after fermentation has started. On the other hand, Thompson and Buddemeyer (4) have demonstrated that calcium stearyl-2 lactylate causes a marked increase in mixing tolerance as determined by the farinograph. Both of these observations have been substantiated using the present flour.

Calcium stearyl-2 lactylate and potassium bromate have improving effects on bread quality which are very similar in their gross aspects. The effects of these two adjuncts are presumably the result of different reactions influencing the colloidal properties of the dough; this is indicated by the extreme difference in the quantitative requirements of the two adjuncts, their additive functions, and the variation in the time of the first observable effect in dough or bread.

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GRAIN STORAGE STUDIES. XX. RELATION BETWEEN VIABILITY, FAT ACIDITY, GERM DAMAGE, FLUORESCENCE VALUE, AND FORMAZAN VALUE OF COMMERCIAL WHEAT SAMPLES[1]

HEINZ SORGER-DOMENIGG, L. S. CUENDET, AND W. F. GEDDES

ABSTRACT

Sixty-eight commercial samples of hard red spring and hard red winter wheats containing from 3 to 60% of germ-damaged kernels and varying in viability from 1 to 95% were analyzed for fat acidity, for the extent of browning as measured by the fluorescence of aqueous extracts, and for dehydrogenase activity (formazan value). Germ damage was positively correlated with fat acidity ($r=0.46$) and with fluorescence value ($r=0.47$), and negatively correlated with viability ($r=0.49$) and with formazan value ($r=0.69$). Viability was negatively correlated with fat acidity ($r=0.92$) and fluorescence value ($r=0.66$) and positively correlated with formazan value ($r=0.60$).

The commercial detection of germ-damaged or "sick" wheat is based on the discoloration of the germ which is observed upon removal of the bran layers which cover it. Visual examinations of this nature are highly empirical; they do not distinguish between different degrees of damage, and in the early stages of deterioration it is difficult to decide whether discoloration has occurred. The researches of Cole and Milner (3) support the view that the tan, brown, or black color of the germs of "sick" wheat are due to a browning reaction of the Maillard type. With a series of wheats, they found a positive correlation of 0.748 between the fluorescence of aqueous extracts of the samples and the federal inspectors' evaluation of germ damage; a significant negative correlation of 0.775 was obtained between fluorescence and the percentage germination of the samples. Cole and Milner suggested that, with additional refinements, the fluorescence test may be useful for the quantitative evaluation of sick wheat and also as an index of viability.

Several workers have proved that germ damage in wheat is associated with high fat acidity (2,5,6,9,10) and with low viability (2,6,9). Recently, Sorger-Domenigg *et al.* (8) reported that losses in viability preceded the discoloration of the germ and were indicative of incipient damage and poor storage properties. This finding led Peterson (7) to investigate the measurement of dehydrogenase activity as a means of

[1] Manuscript received August 23, 1955. Contribution from the Department of Agricultural Biochemistry, Institute of Agriculture, University of Minnesota, St. Paul, Minnesota. Paper No. 3398 of the Scientific Journal Series.

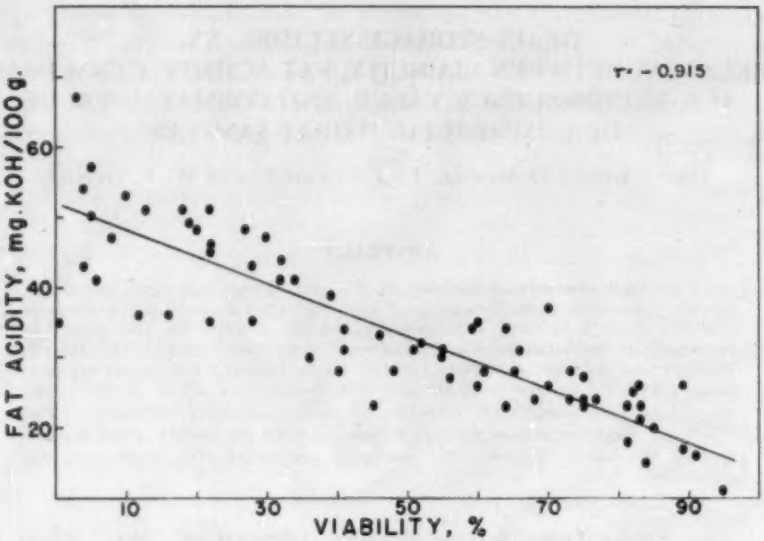

Fig. 1. Relation between viability and fat acidity for 68 samples of commercial hard red spring and hard red winter wheats.

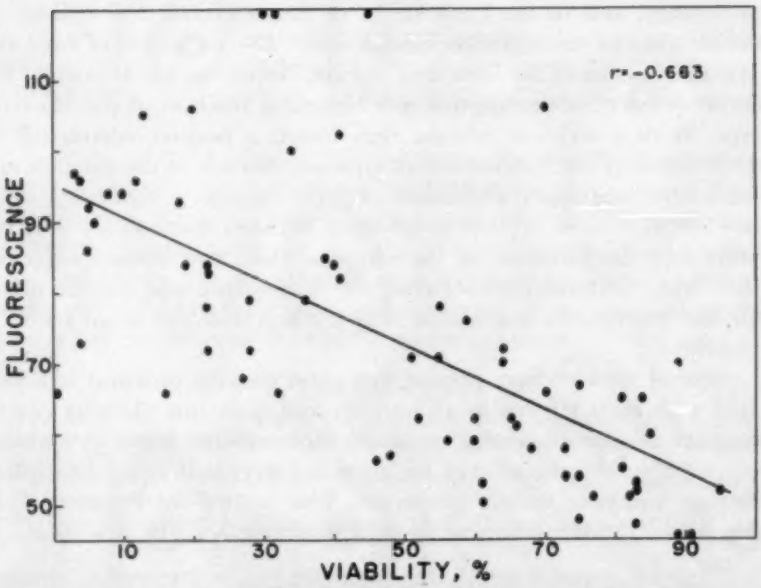

Fig. 2. Relation between viability and fluorescence value for 68 samples of commercial hard red spring and hard red winter wheats.

estimating viability. The estimation is based on the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to formazan, a red-colored compound, which is extracted from the wheat grain and determined colorimetrically. While limited tests showed that the relation between viability and formazan value varied with the variety of the wheat, the location of growth, and the degree of mold infestation, no experiments were conducted with commercial wheats.

The present study was undertaken to determine the relationship between viability, fat acidity, fluorescence value, and formazan value in commercial samples of wheat.

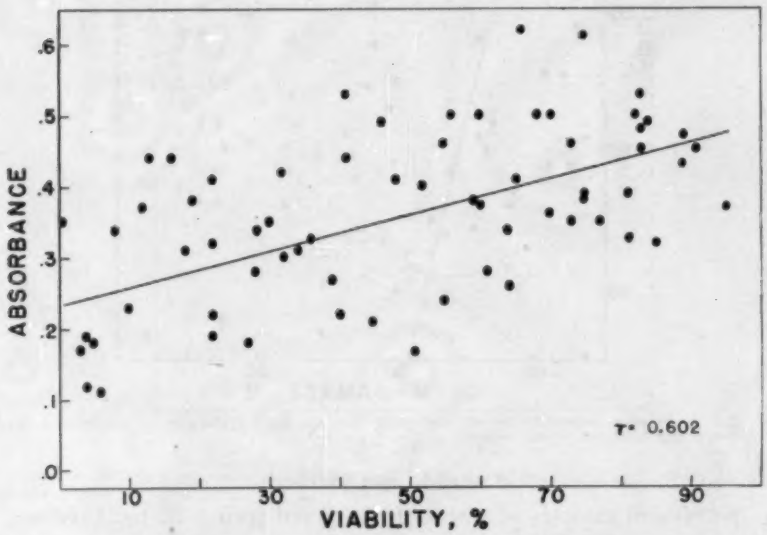

Fig. 3. Relation between viability and formazan value (absorbance) for 68 samples of commercial hard red spring and hard red winter wheats.

TABLE I

SUMMARY OF ANALYTICAL DATA ON SIXTY-EIGHT SAMPLES OF COMMERCIAL HARD RED SPRING AND HARD RED WINTER WHEATS

Property	Minimum	Maximum	Standard Deviation	Mean
Viability, %	1	95	28.0	47.5
Germ damage, %	3	60	14.0	16.6
Fat acidity, mg. potassium hydroxide per 100 g.d.m.	13	67	11.6	33.9
Fluorescence value	43	120	19.3	73.8
Formazan value, absorbance	0.11	0.62	0.12	0.36

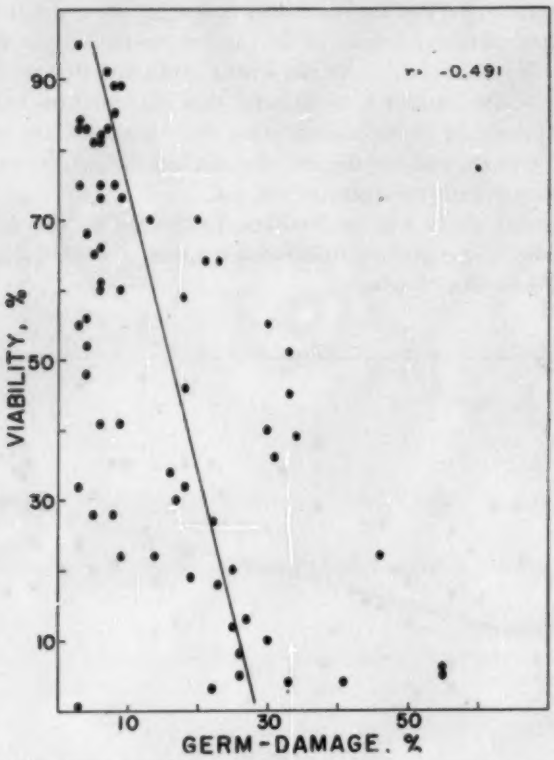

Fig. 4. Relation between viability and germ damage for 68 samples of commercial hard red spring and hard red winter wheats.

Materials and Methods

Sixty-eight samples of wheat (16 hard red spring, 52 hard red winter), each weighing approximately 200 g., obtained from samples of car lots taken for grading, were secured from the Federal Grain Supervision office, Agricultural Marketing Service, in Chicago. Information was provided on the commercial grade and percent germ damage. The samples were stored in closed containers at 4°C. for several weeks before the analyses were made.

Viability was determined by the Minnesota State Seed Testing Laboratory. Two hundred seeds were placed on wet blotting paper supported by wire trays in a germinator maintained at 20°C. The seeds which had normal sprouts after 7 days were considered viable.

Fat acidity was determined on a diethyl ether extract (6 hours) of 6.0 g. of ground meal (micro Wiley mill, No. 40 sieve) according to the method of Ames and Licata (1) as modified by Hunter *et al.* (4). The

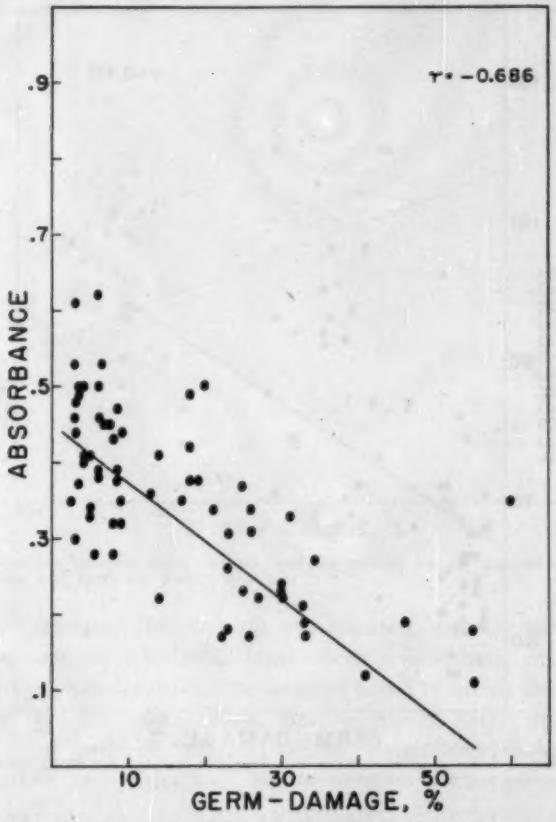

Fig. 5. Relation between germ damage and formazan value (absorbance) for 68 samples of commercial hard red spring and hard red winter wheats.

dried solvent was evaporated in an atmosphere of nitrogen under reduced pressure, the residue dissolved in a benzene-isopropanol mixture (1 + 1 by volume) and titrated with 0.01 *N* potassium hydroxide solution in isopropanol, using phenolphthalein as indicator. The results were expressed as mg. potassium hydroxide per 100 g. dry wheat.

The fluorescence value was obtained by a modification of the method of Cole and Milner (3), which involves measurement of the fluorescence of a wheat extract obtained by shaking 5.0 g. of ground wheat with 0.2 *N* hydrochloric acid solution and subsequent treatment with chloroform to remove dispersed protein material. The fluorescence measurements were made on the undiluted extracts with the Coleman Electronic Photofluorometer and the B1 and PC1 filters. Sodium fluorescein (0.1 p.p.m.) and B2 and PC2 filters were used to

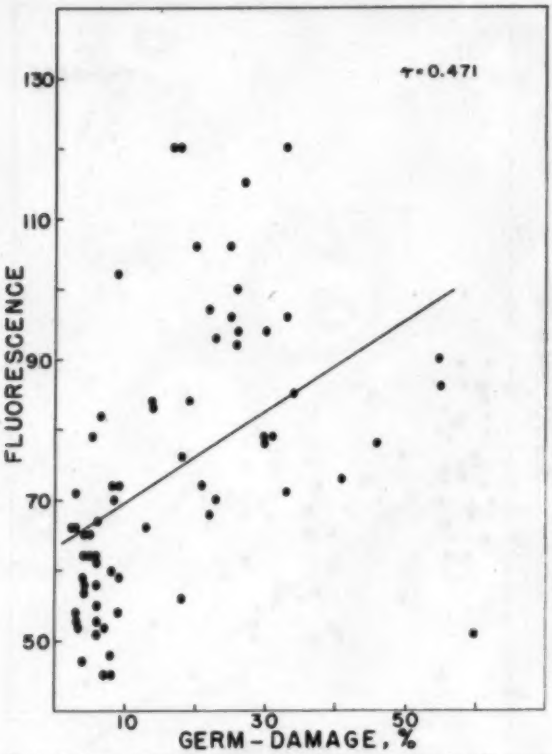

Fig. 6. Relation between germ damage and fluorescence value for 68 samples of commercial hard red spring and hard red winter wheats.

standardize the instrument, the dial being set at 60 with this solution.

The reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to colored formazan by wheat samples was estimated as follows: 3-ml. portions of 3.0% TTC solution (buffered with 0.2 *M* phosphate at pH 7.2) were added to 3-g. samples of wheat and placed in a vacuum oven maintained at 37°C. Reduced pressure was maintained by a water aspi-

TABLE II

SIMPLE CORRELATION COEFFICIENTS BETWEEN VARIOUS PROPERTIES OF SIXTY-EIGHT COMMERCIAL WHEAT SAMPLES [a]

	Fat Acidity	Fluorescence Value	Formazan Value	Germ Damage
Viability	−0.92	−0.66	+0.60	−0.49
Fat acidity		+0.67	−0.56	+0.46
Fluorescence value			−0.44	+0.47
Formazan value				−0.69

[a] Value of r at 1% point = 0.30.

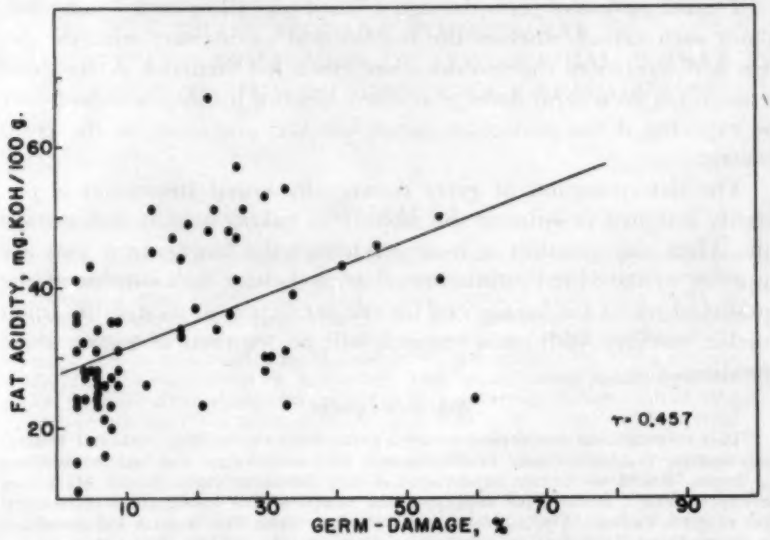

Fig. 7. Relation between germ damage and fat acidity for 68 samples of commercial hard red spring and hard red winter wheats.

rator for 30 minutes, the vacuum was released, and the samples were kept in the oven an additional hour under atmospheric pressure. The excess solution was decanted, the samples dried in an air oven at 80°C. for 10 minutes, and the ground samples (micro Wiley mill, No. 20 sieve) extracted with 40 ml. acetone by continuous shaking for 30 minutes. After centrifugation, the absorbance was read at a wave length of 520 mμ on a Coleman Junior Spectrophotometer.

Results and Discussion

The results are summarized by the statistical constants in Table I, the scattergrams in Figures 1 to 7, and the simple correlation coefficients in Table II. The correlation coefficients between germ damage and fluorescence value (+0.47) and between germ damage and viability (−0.49) are lower than those reported by Cole and Milner; namely +0.75 and −0.78 respectively. Although the correlation of −0.69 between germ damage and formazan value is somewhat higher than those involving viability, fat acidity, and fluorescence value, none is of sufficient magnitude to justify its use for predicting the extent of germ damage as determined by grain inspectors. Viability, likewise, cannot be accurately estimated from the fluorescence value, formazan value, or the extent of germ damage. However, viability is highly correlated with fat acidity (−0.92).

The estimation of germ damage is based on "all or none" judgment about each kernel, whereas the biochemical values vary with the extent and severity of the deterioration which has occurred. A high correlation between germ damage and any biochemical factor would only be expected if the particular factor was the sole cause of the germ damage.

The determination of germ damage by visual inspection is primarily designed to estimate the damage to baking quality and storage life. Thus, the question at issue is whether the biochemical tests are superior to the visual estimation of germ damage as an index of the quality of wheat for baking and for the prediction of its stability upon further storage. Additional research will be required to answer these questions.

Acknowledgment

This investigation was supported by a grant from the Millers' National Federation and the National Grain Trade Council. The authors are indebted to William L. Ingles, Board of Grain Supervisors, Grain Division, Agricultural Marketing Service, Chicago, Illinois, for supplying the samples with information on the extent of germ damage. The authors are indebted to Anne Peterson for her assistance in determining the formazan values.

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GRAIN STORAGE STUDIES. XXI. VIABILITY AND MOLDINESS OF COMMERCIAL WHEAT IN RELATION TO THE INCIDENCE OF GERM DAMAGE[1]

CLYDE M. CHRISTENSEN[2]

ABSTRACT

Germination of the seed, and number and kinds of molds present, were determined in "sick" and sound seeds picked from 26 commercial samples containing from 5–55% "sick" wheat, and in sound wheat from bulks in which no deterioration had occurred. The germination of "sick" seed always was zero; molds were microscopically visible on the germs of 49% of the seeds; they had an average mold count of 402,000/g; and 94% of the surface-disinfected seeds yielded storage molds. The "sound" seeds picked from the lots in which deterioration had occurred had an average germination of 43% and an average mold count of 32,000/g, and 84% of the surface-disinfected seeds yielded storage molds. The really sound seeds from bulks in which no deterioration had occurred had an average germination of 91%, an average mold count of less than 1,000/g, and 27% of the surface-disinfected seeds yielded storage molds. The major fungi present in the "sick" seeds were *Aspergillus restrictus, A. repens, A. candidus,* and *A. flavus.* Judged by various microscopic and cultural technics, all samples of "sick" wheat had been very heavily invaded by storage molds; all of the evidence indicated that invasion of the germs of the seeds of these molds had preceded decrease in germination and increase in "sick" wheat. In commercial storage, it seems very probable that invasion of the germs of the seed by common species of *Aspergillus* is a common cause of "sick" wheat.

Various laboratory studies have shown that the germs of wheat may turn brown as a result of invasion by molds (4, 5, 9), exposure to high temperature and high moisture in the presumed absence of molds (7), or storage under high carbon dioxide concentrations at moisture contents above 15% (8). There have been few studies of wheat that has become "sick" in commercial storage, and thus it is not known which conditions resulting in brown germs in the laboratory duplicated the processes that lead to the development of "sick" wheat in bulk stored grain. Such evidence as is available, however, on samples from commercial bins, strongly indicates that invasion of seed by molds precedes the development of "sick" wheat (4, 5, 9). To evaluate the processes that might have led to the development of "sick" wheat in commercial bins, and to furnish a basis for studies that might duplicate these in the laboratory, it seemed desirable to investigate some of the microbiological characteristics of wheats that have deteriorated in commercial storage, and to compare these with wheats in which no deteriora-

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tion had occurred. The present report summarizes data obtained from a study of "sick" and sound seeds selected from 26 commercial samples containing various amounts of "sick" wheat, and from 12 samples of wheat in which no deterioration had occurred.

Materials and Methods

Samples. Samples containing "sick" wheat were obtained during the fall of 1954 from the Grain Division, Agricultural Marketing Service, Chicago, Illinois, from commercial parcels being inspected at that time. The majority were hard red winter wheats, but a few were dark northern spring. The amount of "sick" wheat in the samples ranged from 5 to 55%. Samples in which no deterioration had occurred were obtained from a commercial bin in Enid, Oklahoma; they were of the 1954 crop, stored since early summer of 1954. Samples were obtained by probe from depths of 20 and 30 feet in several different places in the bin, early in February and again in May, 1955. The samples were sent to St. Paul in screw-capped tins equipped with new waxed liners in the caps, and were tested for moisture content, germination, and mold invasion within a few hours after they arrived.

Moisture Content. Moisture content was determined by the two-stage, air-oven method specified in *Cereal Laboratory Methods* (1), and is expressed on a wet-weight basis. Moisture contents were determined only on the samples from Enid, Oklahoma, since the samples from Chicago had been exposed to air during inspection and subsequent shipment to us.

Separation of "Sick" and "Sound" Seed. The seeds were examined individually with a stereoscopic microscope under a magnification of ×10. Pericarps were removed from the germs, and the germs were inspected with the microscope. Seeds with obviously dark germs were considered "sick," while those with white or only slightly discolored germs were considered "sound." The percentage of "sick" seed was determined by counting and by weighing. In most cases, from 100 to 200 "sick" seeds were selected, which usually involved examining somewhat more than 10 g. of seed, the amount normally used by grain inspectors in determining percentage of sick wheat.

Molds Visible on Germ Surface. The percentage of seeds with molds visible on the germ surface was determined by examining the germs of 100 seeds with the stereoscopic microscope, ×10, after the pericarps had been removed from the germ.

Germination. Fifty "sick" and 100 "sound" seeds from each sample were tested for germination. The seeds were placed on moist paper, rolled loosely, wrapped in waxed paper, and kept in a large covered

dish at room temperature. Germination percentage was determined after 4 to 6 days. Not all samples could be left for 6 days because many of the supposedly "sound" seeds with relatively low germination were so heavily overgrown by molds within 4 days that the germinated and nongerminated seeds could be separated only with difficulty.

Mold Count. Mold counts were made by comminuting seeds in 500 ml. of a 0.2% sterile solution of agar in water, in a Waring Blendor for 1½ minutes, suspending 5 ml. of this in 45 ml. of sterile 0.2% agar solution, and culturing 1-ml. replicate aliquots in malt agar containing 7.5% sodium chloride. From the lots in which deterioration had occurred, only 50 "sick" and 50 "sound" seeds were used; from those lots in which no deterioration had occurred, 5 g. of seed were used. In some of the tests a suspension medium containing 0.2% agar and 7.5% sodium chloride was used, because this sometimes yielded higher counts of *Aspergillus restrictus* than did a suspension medium containing no sodium chloride. At the beginning of the study the comminuted suspension of "sick" and "sound" seeds picked from the lots in which deterioration had occurred were cultured in agar media favorable to the growth of bacteria. In all of these, the number of bacteria obtained was lower than the number reported from sound wheat (6). This supported unpublished evidence from tests made at various times in these laboratories, to the effect that bacteria are not normally concerned in the type of deterioration here investigated, and for this reason no further tests for bacteria were made.

Molds from Surface-Disinfected Seeds. Fifty "sick" and 100 "sound" seeds picked from those samples in which deterioration had occurred, and 100 "sound" seeds from those lots in which no deterioration had occurred were shaken for 30 seconds in a solution containing approximately 250 p.p.m. of available chlorine, rinsed twice in sterile water, and cultured on malt agar containing 7.5% sodium chloride.

Results

The principal data are summarized in Tables 1 and 2.

Determination of Amount of "Sick" Wheat. Determination of amounts of "sick" wheat by count and by weight gave essentially the same results in nearly all cases, and therefore only the figures obtained by weight are given. The amount of "sick" wheat determined by the writer averaged 2% higher than that determined by the Chicago grain inspectors. In some lots, the germs, after the pericarps had been removed, were covered with a whitish mold. Inspected only with the naked eye, or with low magnification such as afforded by a tripod lens, it is doubtful if one not familiar with molds would recognize this as

TABLE I

GERMINATION AND MOLD INVASION OF "SICK" AND "SOUND" SEEDS FROM COMMERCIAL SAMPLES IN WHICH DETERIORATION HAD OCCURRED

SAMPLE NO.	"SICK"	WITH MOLDS VISIBLE ON GERMS		GERMINATION[a] OF SOUND SEEDS	MOLD COUNT PER GRAM (IN THOUSANDS)		SURFACE-DISINFECTED SEEDS YIELDING STORAGE MOLDS	
		"Sick"	Sound		"Sick"	Sound	"Sick"	Sound
	%	%	%	%			%	%
1	8	70	1	75	507	24	100	100
2	10	74	2	83	904	72	100	86
3	10	50	0	92	273	11	100	80
4	11	39	0	86	217	13	100	76
5	12	70	3	92	653	72	100	100
6	17	37	0	22	100	45	82	60
7	18	31	5	12	240	1	98	98
8	18	52	0	20	1560	1	100	84
9	20	92	1	74	3	6	100	100
10	22	27	0	10	1	1	94	58
11	22	80	10	36	900	30	68	26
12	25	56	1	16	1140	6	100	96
13	30	52	1	38	211	20	100	100
14	30	65	8	75	410	27	100	53
15	30	53	1	90	806	17	100	100
16	31	57	12	59	1081	153	100	100
17	31	52	1	34	1032	3	100	100
18	33	55	1	74	132	28	100	100
19	33	49	3	46	210	39	100	100
20	34	75	4	84	480	154	100	100
21	40	27	0	0	18	14	56	32
22	41	19	1	6	48	5	94	90
23	44	61	1	40	31	5	58	58
24	50	63	7	58	430	70	100	100
25	55	50	4	20	4	4	98	88
26	55	43	2	0	105	18	96	86
Mean	29	49	3	43	402	32	94	84

[a] The germination of "sick" seeds was 0 in all samples.

TABLE II

MOISTURE CONTENT, GERMINATION, AND MOLDINESS OF TWELVE SAMPLES OF COMMERCIAL WHEAT IN WHICH NO DETERMINATION HAD OCCURRED

SAMPLE NO.	MOISTURE CONTENT	GERMINATION	SURFACE-DISINFECTED SEEDS YIELDING STORAGE MOLDS[a]
	%	%	%
1	11.3	76	30
2	11.4	87	10
3	11.3	88	14
4	11.5	88	38
5	11.6	89	28
6	11.4	89	12
7	11.5	94	14
8	11.3	94	32
9	11.5	95	22
10	11.3	95	16
11	11.3	96	16
12	11.7	97	94
Mean	11.4	91	27

[a] The mold count was less than 1000/g in all samples.

mold, and such seeds probably would be classified as "sound." Also it is possible that the writer was slightly more strict than the inspectors in evaluating seeds as "sick." However, the agreement in most cases was very close, the largest difference being 11%.

Molds Visible on Surface of Germs. The seeds of most of the lots in

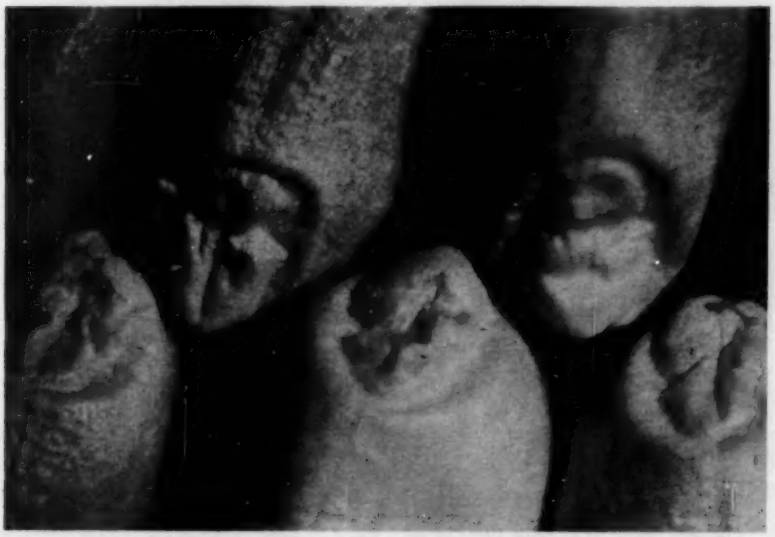

Fig. 1. External view of seeds from a parcel of hard red spring wheat containing 30% "sick" seeds. No molds are visible on the outside of the seeds.

which deterioration had occurred, externally appeared to be mold-free. Small tufts of sporophores of *Aspergillus* could be seen protruding from the germ end of a few seeds of a few samples, but only in those containing a high percentage of "sick" wheat. The outside of the seeds of nearly all samples appeared about as shown in Fig. 1.

When the pericarps covering the germ were removed, and the seeds examined with the stereoscopic microscope, using magnifications of ×10 and ×20, the germs of many of the "sick" seeds were found to be exceedingly moldy. Typical cases are shown in Figs. 2 and 3. Not uncommonly, the molds were so heavy as to conceal the germ, and in some samples masses of perithecia of *Aspergillus glaucus* covered the germs (Fig. 4). Molds were visible on the germs of an average of 49% of the "sick" seeds, with a range of from 19 to 92%. Light molding was visible on the surface of the germs of 3% of the "sound" seeds picked from the lots in which deterioration had occurred; the germs of all of these were slightly discolored, but not dark enough to be considered "sick." In a number of samples a thin crust of mold was present on the outside of the pericarp over the germs of "sick" seeds. With a microscope, this was readily visible as mycelium, but to the naked eye it appeared only as a dull gray area.

Some of the "sick" seeds, especially those with dark brown to black germs, appeared to be mold-free when examined with a magnification

Fig. 2. Typical "sick" seeds from the same parcel as those shown in Fig. 1. The pericarps were removed to expose the germ; molds were visible on the surface of 70% of the "sick" seeds.

of ×10, a typical one being illustrated in Fig. 5. When such seeds were cultured on malt-salt agar, various subspecies of *Aspergillus glaucus* grew from the germs of 100% of those which were not surface-disinfected, and from 60% of those which were surface-disinfected, as shown in Fig. 6. Some of the "sick" seeds selected as having no mold visible on the germ, and which yielded no molds when cultured after surface-disinfection, were studied by examining the germs under a magnification of ×100 to ×400. Masses of mycelium, as shown in Fig. 7, were

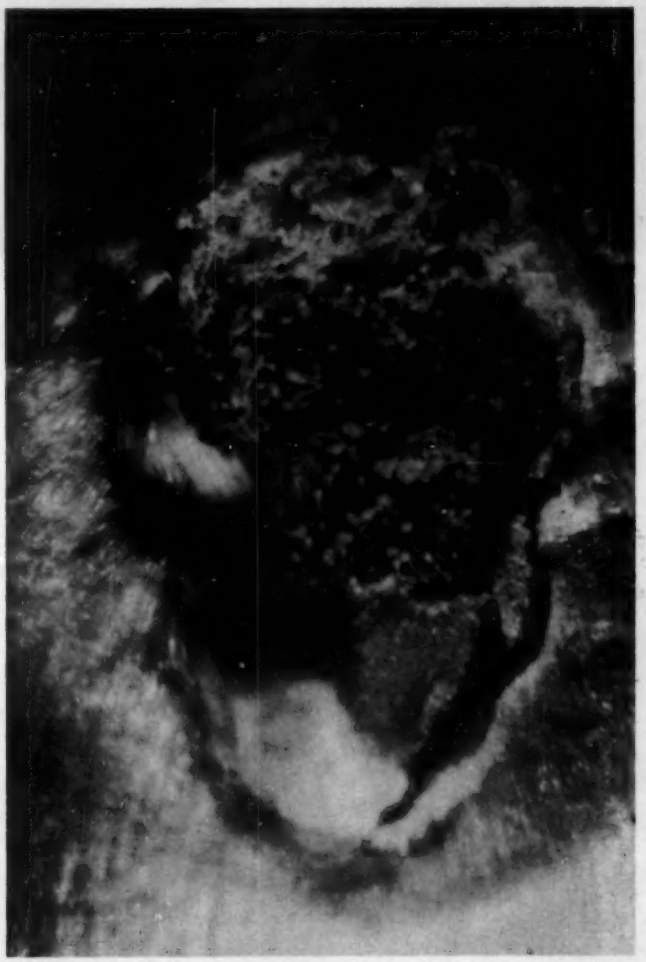

Fig. 3. Seeds of "sick" wheat from a commercial lot of hard red winter wheat, with the pericarps removed to expose the germ. Most of the germ is covered with a dense growth of Aspergillus glaucus.

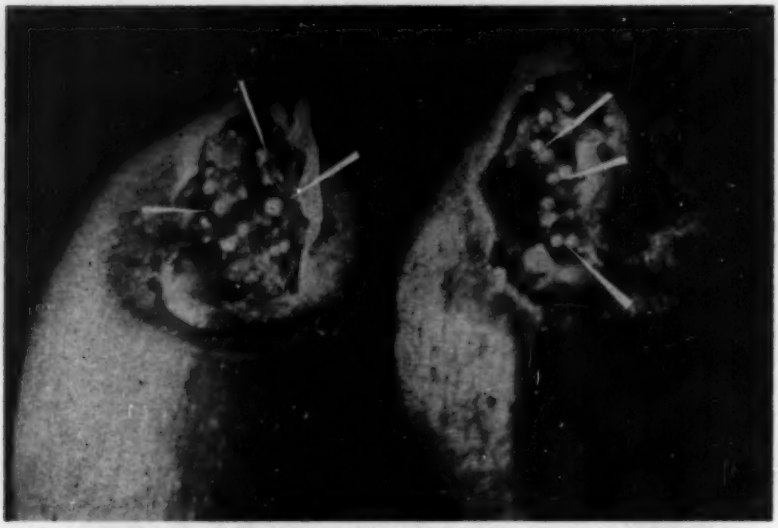

Fig. 4. Seeds of 'sick" wheat with perithecia (indicated by arrows) of Aspergillus glaucus on the surface of the germ.

Fig. 5. Sample No. 25. "Sick" seed selected as having no mold visible on the exposed germ when examined under a magnification of ×10 and ×20. Seeds similar to this are shown, after culturing, in Fig. 6, and the surface of the germ in Fig. 7.

found on the surface of every germ so examined.

No molds were visible on or within the germs of any of the seeds from Enid, Oklahoma, in which no deterioration had occurred (these were also examined at magnifications of ×100 and ×400), and no discoloration was evident in any of the germs.

Germination. In all samples, the germination of "sick" seeds was zero, and it can only be concluded that "sick" seed is dead. The germination of "sound" seeds from the samples containing various amounts of "sick" seed ranged from zero to 92%, and averaged only 43%. This suggests that decrease in germination precedes the appearance of "sick" wheat, which is in agreement with the results from previous studies (4, 5, 9). In samples 1 through 5, however, which contained 8–12% "sick" seed, the average germination of the "sick" and "sound" combined was above 75%, indicating that some "sick" seed

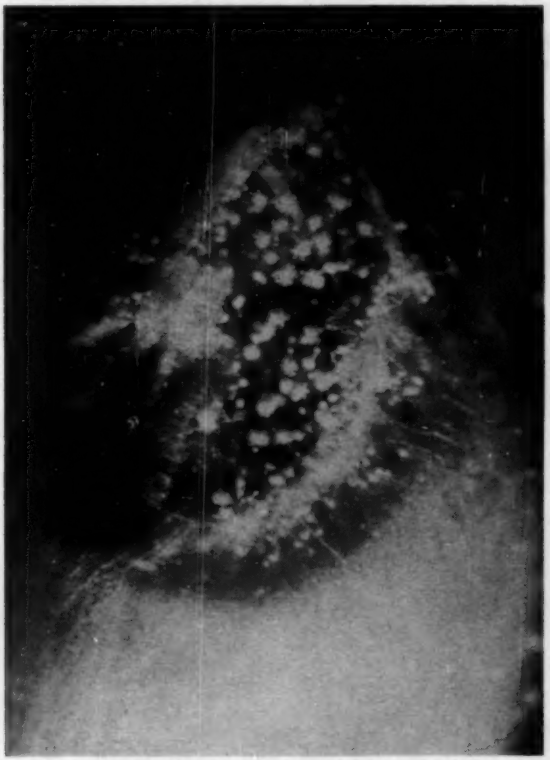

Fig. 6. Sporophores of Aspergillus glaucus growing from the germ of a seed of "sick" wheat which had no molds visible on the surface of the germ. The seed was surface-disinfected, cultured on agar, and incubated for several days before it was photographed.

may be present in lots with relatively high germination.

Germination of the seeds in the twelve lots in which no deterioration had occurred averaged 91%.

Mold Count. The "sick" seeds averaged 402,000 molds per g., which is, according to other evidence (2, 3) exceedingly moldy wheat. In nearly all cases the major portion of the count consisted of subspecies of *Aspergillus glaucus,* although *A. candidus* and *A. flavus* predominated in a few samples. *A. restrictus,* a subspecies of *A. glaucus* known to invade wheat and barley at moisture contents of 14% or slightly below (4, 10), was prevalent in over half the samples, indicating that this fungus may be of considerable practical significance in the development of sick wheat in commercial bins.

The "sound" seeds from lots in which deterioration had occurred had an average mold count of 32,000 per g., with a range of from 1,000 to 154,000 per g. Judged by this criterion, as well as by germination, many of these "sound" seeds had undergone considerable deterioration even though the germs had not yet become discolored.

Both "sick" and "sound" seeds from a few lots in which considerable deterioration had occurred gave a low mold count. In sample No.

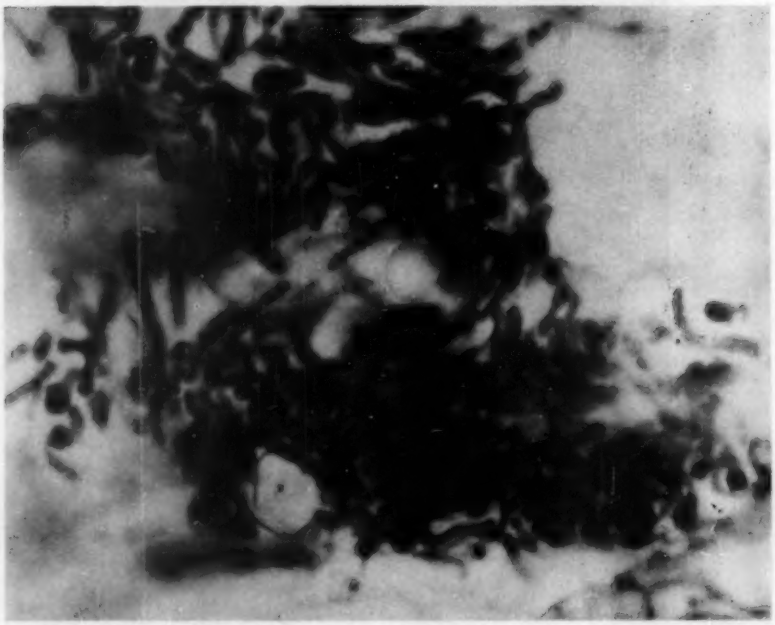

Fig. 7. Mold mycelium on the surface of a germ of "sick" wheat. No mold was visible on the germ of this seed when examined with a magnification of ×10 or ×20, and no mold grew from the seed when it was cultured.

9, for example, mold was visible on the germs of 92% of the "sick" seeds, often so heavy as to conceal the germs, yet the mold count was only 2,000 per g. Obviously the abundant mold (apparently *Aspergillus restrictus*) present on the germs was dead. The reason for this could not be determined. However, the "sick" seeds in 20 of the 26 samples had a mold count of 100,000 or more per g., additional evidence that "sick" wheat is moldy.

The mold count of twelve lots of sound wheat from Enid, Oklahoma, in which no deterioration had occurred, averaged less than 1000 per g. (Table II).

Molds from Surface-Disinfected Seeds. When surface-disinfected and cultured on malt-salt agar, both "sick" and "sound" seeds from lots in which deterioration had occurred yielded *Aspergillus glaucus*, especially the subspecies *A. repens*, from nearly every seed. Some lots also yielded *A. candidus* and *A. flavus* from a considerable number of both "sick" and "sound" seeds. In sample No. 8, for example, 60% of the "sound" seeds after surface disinfection yielded *A. glaucus*, 10% *A. candidus*, and 14% *A. flavus*. In sample No. 13, 100% of the "sound" seeds after surface disinfection yielded *A. glaucus*, 64% *A. candidus*, and 25% *A. flavus*. The "sound" seeds of samples 1 through 5, which had the highest germination of any of the lots in which deterioration had occurred, after surface disinfection yielded *A. glaucus* from 76 to 100%, and *A. flavus* from 12 to 70%. This again is fairly conclusive evidence that, in commercial storage, invasion of the seeds by storage molds precedes decrease in germination and development of "sick" wheat.

The twelve lots in which no deterioration had occurred yielded storage molds from 27% of the surface-disinfected seeds (Table II). In all of these the invasion appeared to be relatively light.

In general, the number and kinds of molds obtained from the commercial parcels containing "sick" seeds were within the range of those recently reported by Sorger-Domenigg *et al.* (9). Apparently their tests, in which "sick" wheat appeared in grain that had been invaded by molds in the laboratory, duplicated fairly closely the conditions under which the samples studied here became "sick" in commercial storage. From this it seems reasonable to suppose that "sick" wheat commonly is a product of invasion of the germs of stored seeds by molds.

Acknowledgments

This study was supported in part by a grant from the Millers' National Federation and the National Grain Trade Council. The writer is indebted to Wm. L. Ingles, Board of Grain Supervisors, Grain Division, Grain Marketing Service, Chi-

cago, Illinois, for most of the samples containing damaged seed, and for data on the amount of germ damage they contained when inspected.

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EFFECTS OF STORAGE TEMPERATURE AND FREEZING ON THE FIRMING OF A COMMERCIAL BREAD[1]

JAMES W. PENCE[2] AND NOEL N. STANDRIDGE[2]

ABSTRACT

Firming in bread crumb, measured as force per fixed deformation on whole slices, became progressively more rapid at storage temperatures of 86°, 74°, 54°, and 46°F. (30°, 23°, 12°, and 8°C.). At 34°F. (1°C.), however, firming was slightly more rapid than at 20°F. (—7°C.) and 46°F. (8°C.). Comparisons of firming rates at the lower temperatures were difficult after the first day or two because, after appreciable firmness had developed, differences between loaves on the same day were often as large or larger than those between loaves on successive days.

At 86°F. (30°C.) and 74°F. (23°C.), firmness increased almost linearly with time, but at the lower temperatures the relationship was definitely curvilinear. At the four lower temperatures used, approximately half of the total increase in firmness up to the sixth day after baking had occurred within the first 24 hours.

Firming in bread that was frozen and thawed before storage was slower than in other loaves of the same lot of bread stored without preliminary freezing; firming in the defrosted bread was compared to that in unfrozen bread starting at the same level of firmness. The freezing and thawing (90 minutes at —20°F. or —29°C. and 5 hours at 74°F. or 23°C.) — caused an amount of firming approximately equal to 24 hours of storage of unfrozen bread at 74°F. (23°C.), but 48 hours after defrosting, the frozen bread was equal in firmness to the unfrozen bread at 48 hours of age.

One of the primary objectives of freezing bread commercially is to minimize all staling changes, including firming, as much as possible. The freezing and defrosting of bread unavoidably cause an increase in its firmness, and the extent of this increase depends on how rapidly the processes are carried out. A critical evaluation of the practical value of freezing bread, therefore, requires a corresponding knowledge of how fast unfrozen bread becomes firm under conventional handling.

Previous workers (3, 7, 8, 12, 13) have shown that bread firms faster as its temperature is lowered toward the freezing point, and that moderate changes in storage temperature can cause commercially significant difference in firmness. Meisner (8), for example, found large differences in the decline of bread softness at temperatures of 30°, 40°, 75°, and 110°F. (—1°, 4°, 24°, and 43°C.).

In an investigation of the effects of freezing, frozen storage, and defrosting of bread in progress at this laboratory (9), a study of the effect of temperature on firming in bread was included to provide for a direct comparison of the amount of firmness caused by freezing with

[1] Manuscript received May 17, 1955. Presented at the Annual Meeting in St. Louis, Missouri, May 1955. Contribution from Western Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Albany 10, California.

[2] Western Utilization Research Branch, Albany 10, California.

that which would normally occur in unfrozen bread at various temperatures. The firmness results obtained in the latter case are being presented to supplement the softness data of Meisner (8) and others because values obtained with fixed force (softness) are reported to be difficult to convert directly to values obtained with fixed deformation (firmness) (2, 5). Firming rates of unfrozen bread were also compared with those for bread frozen and defrosted before storage, a question about which there has been much recent speculation.

Materials and Methods

The bread used in these experiments was unsliced, commercial white pan bread wrapped in the customary waxed paper and obtained from a single bakery about 2 hours after baking. It was immediately placed in storage at selected temperatures which were maintained well within a ±2°F. range except in the case of room temperature where the deviations equaled the full extent of this range. Temperatures at the center of the test loaves equaled the storage temperatures within 3 to 6 hours after they were placed in the storage compartments.

Firmness readings were made with a Baker Compressimeter,[3] equipped with a pressure plate 36 mm. ($1\frac{7}{16}$ in.) in diameter, on slices cut ¾ in. thick by means of a miter box. Slices were individually sealed in cellophane bags and allowed to come to room temperature before firmness was measured.

Hand-sliced bread was used in these studies in order to obtain a direct comparison with other studies on frozen bread (9, 10). A slice thickness of ¾ in. (19 mm.) was selected to minimize possible variation in slice thickness due to hand slicing. The firmness values obtained, therefore, might be expected to be slightly lower than for the usual ½-in. (13 mm.) thickness in commercially sliced bread, but measurements made for both slice thicknesses in bread at 2 to 3 and 24 hours of age agreed closely. Crossland and Favor (5) found significant differences in firmness values when larger differences in slice thickness (12 mm. vs. 30 mm.) were compared, but the difference in slice thickness did not alter the nature of firmness curves.

Bread that was frozen and defrosted before storage was frozen in approximately 90 minutes at −20°F. (−29°C.), equilibrated overnight or stored for a time at 0°F. (−18°C.), and then allowed to defrost at the temperature at which it was subsequently stored. A period of 4 to 6 hours was required for the frozen bread to reach the final storage temperature. Firmness readings were made immediately after defrost-

[3] Mention of trade names or equipment does not constitute endorsement by the Department of Agriculture over others of a similar nature not mentioned.

ing to establish a base point for comparison of firming in frozen-and-defrosted and unfrozen bread.

Firmness values were determined as averages for ten slices from the center section of each of three loaves for each storage interval at each temperature. All storage experiments were run in duplicate on separate occasions and with different lots of bread to make the selection of bread more nearly random. Whole slices were used for firmness readings, and no attempt was made to avoid the effect of shear on the firmness measurements.[4] Readings were taken for both 4 and 5 mm. of compression, but the results were parallel. Crumbliness of a few samples of stored bread was measured by the modified sieve-abrasion method of Bradley and Thompson (4).

Results and Discussion

Effect of Temperature. The curves in Fig. 1 show the effect of temperature on development of firmness in bread stored 6 days. The firming was progressively more rapid for each decrease in temperature down to 46°F. (8°C.), but at 34°F. (1°C.) it was slightly faster than at 20°F. (−7°C.) and 46°F. (8°C). This is more clearly illustrated in Fig. 2 where rate of firming is plotted against temperature. The points on the curve are arbitrary averages at each temperature for all points on Fig. 1 falling between 60 and 140 hours where the lines are most nearly linear. The peak in the curve in Fig. 2 shows the maximum

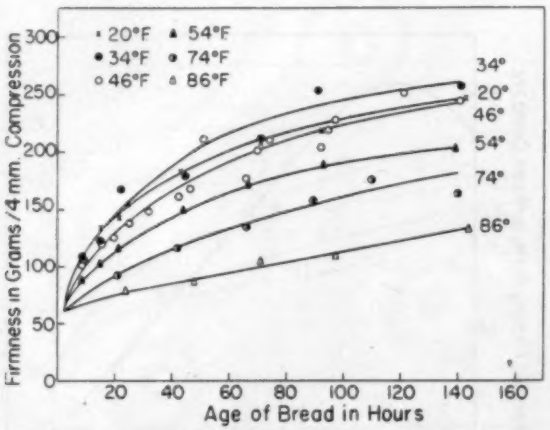

Fig. 1. The effect of temperature on firming of a commercial white pan bread. The temperatures used correspond to 30°, 23°, 12°, 8°, 1°, and −7°C.

[4] Crossland and Favor (3) showed that the ratio of force per fixed compression (firmness) in prisms of crumb equal in area to the compressimeter plunger and in whole slices was the same for a compression of 4 mm. up to at least the third day of storage.

estimated firming rate at 34°F. (1°C.). A significant difference in the firming rates shown for the three lower temperatures is doubtful, because comparisons were difficult after appreciable firmness had developed in the bread. Differences between loaves on the same day were often as large as, or larger than, those between loaves on successive days. However, it is interesting to note that the greatest apparent rate of firming occurred at a temperature close to the 2° to 3°C. (36° to 37°F.) stated by Katz (7) to be the temperatures for the maximum rate of staling in bread.

At 86° and 74°F. (30° and 23°C.), firmness increased almost linearly with time, but at the lower temperatures the relationship was curved. Similar observations were reported by Steller and Bailey (13) for comparable storage conditions. Even at 80°F. (27°C.), Edelman, Cathcart, and Berquist (6) found curved relationships during the first day or two of storage. These findings emphasize that the firming of bread crumb is a complex phenomenon and that simplifications in its description must be made with caution.

In agreement with results reported by previous workers (6, 7, 8, 13), roughly half the total increase in firmness for the 6 days of storage occurred in the first 20 to 30 hours after baking. The total increase in firmness for 6 days at 86°F. (30°C.) was approximately equal to that for a single day of storage at 46°F. (8°C.) – a temperature near that at which most household refrigerators operate. Housewives who store bread in the refrigerator to retard staling are actually accelerating it

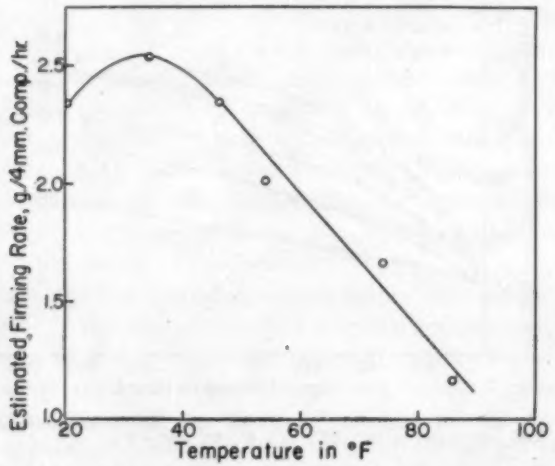

Fig. 2. The relation of temperature to the estimated average rate of firming of a commercial white pan bread. The temperatures used were 30°, 23°, 12°, 8°, 1°, and −7°C.

insofar as firming is concerned. In fact, Bradley and Thompson (3) and Steller and Bailey (13), as well as others, showed that crumbliness and other factors associated with staling also increase more rapidly as storage temperature of bread is lowered.

Effect of Freezing and Defrosting. The firming curves shown in Fig. 3 indicate that bread which has been frozen (90 minutes at —20°F. or —29°C.), stored overnight at 0°F. (—18°C.), and then defrosted (5 hours at 74°F. or 23°C.) becomes firm at a slower rate than unfrozen bread at the same temperatures. Bread stored for 2 weeks at 0°F. (—18°C.) before defrosting gave the same results. The increase in firmness due to freezing and thawing was approximately equal to the gain in firmness of unfrozen bread during about the first 24 hours of storage. However, the firmness of frozen bread 48 hours after defrosting was identical with that of unfrozen bread 48 hours after baking.

These findings are particularly important to those interested in commercial freezing because they show that bread frozen for 2 weeks (and possibly longer) may be somewhat firmer after defrosting yet can have the normal shelf-life expected for fresh bread. In 48 hours the defrosted bread became indistinguishable in firmness from bread 48 hours old that had not been frozen. These relations are illustrated more clearly in Fig. 4 in which firmness values after defrosting are plotted from zero time on the abscissa.

The crumbliness values which follow (Table I) show that this factor also developed more rapidly in the unfrozen bread than in the frozen-

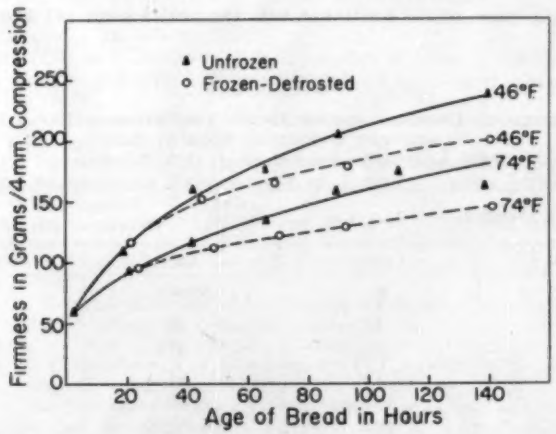

Fig. 3. Comparison of time-firmness curves for unfrozen and frozen-and-defrosted bread, stored at two temperatures, showing differences in apparent rates of firming. The temperatures used were 8° and 23°C.

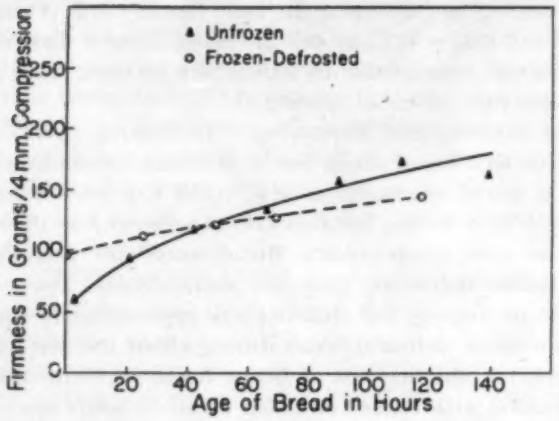

Fig. 4. Comparison of time-firmness curves for unfrozen and frozen-and-defrosted bread stored at room temperature (74°F. or 23°C.), showing differences in anticipated shelf life.

and-defrosted bread during subsequent storage of both at 46°F. (8°C.). With but one exception, the values for frozen-and-defrosted bread are lower than for the corresponding unfrozen samples.

These findings confirm the observations of Anderson (1) that soft rolls and certain types of bread stale more slowly after freezing than the fresh items, so that the practical shelf life of frozen-and-defrosted goods was as long as that of the unfrozen ones, even though the unfrozen items were slightly softer at the beginning of the comparisons. In fact, customer acceptance was greater for some of the frozen items than for unfrozen ones. Earlier, Cathcart and Luber (4) found crumb

TABLE I

CRUMBLINESS OF UNFROZEN AND OF FROZEN-AND-DEFROSTED BREAD DURING SUBSEQUENT STORAGE OF BOTH AT 46°F.
(Grams of crumb passed through U.S. No. 4 sieve)[a]

UNFROZEN BREAD		FROZEN-AND-DEFROSTED BREAD	
Age[b]	Crumb	Age[b]	Crumb
hours	*g.*	*hours*	*g.*
24	3.3	13	1.7
43	7.3	37	5.2
67	7.9	61	8.4
91	12.3	84	7.7
140	18.6	132	12.7

[a] Modified sieve-abrasion method of Bradley and Thompson (4).
[b] Hours after baking for unfrozen bread; hours after defrosting for frozen bread.

compressibility and swelling power to decrease as fast in unfrozen bread as in bread frozen at −22°C. (−8°F.) for 24 hours. Rotsch and Tehsmer (11) also reported recently that frozen rolls and cakes staled[5] less rapidly after defrosting than the same products fresh at the time the frozen materials were defrosted.

No explanation for the reduced rate of firming in defrosted bread can be offered, but the phenomenon may be similar to the refreshening observed by Cathcart and Luber (4) during prolonged storage of bread at −31°F. (−35°C.) These workers found both crumb compressibility and swelling power to pass through a minimum during a 60-day storage period.

Perhaps the most important conclusion from the results presented is that in a practical sense no penalty with respect to quality need be associated with the commercial freezing of bread. However, the process must be properly conducted. The handling of bread both before and after freezing is as important as the procedures of freezing and defrosting on final results. For example, the rapidity with which firmness develops in freshly baked bread emphasizes the importance of freezing bread as soon and as rapidly as possible after baking. The reduced rate of firming in defrosted bread can then compensate to a large extent for the firmness imparted by freezing and defrosting.

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BOOK REVIEWS

The Measurement of Particle Size in Very Fine Particles, by H. E. Rose. Chemical Publishing Co., New York, 1954, 127 pp. Price, $2.75.

This booklet consists of a series of four lectures given by the author at University of London.

Lecture I deals with particle size and size distribution in a general way, and presents some data on mean particle size based on different diameters of certain commercial products.

Lecture II discusses measurement of particle size distribution by sedimentation and elutriation procedures. This lecture is essentially a review of literature on certain classical methods in micromeritics, but does not cover the many variations found in the more recent literature.

Lecture III treats of optical extinction principles in connection with particle size studies. This appears to be an interesting and important contribution, since so little is available in the literature. A second part of this lecture deals with permeability measurements of specific surface and corrections for variations in void content to obtain a "true" surface. Treatment is somewhat cursory since the flow through packings is a rather involved subject, as emphasized by the author.

Lecture IV describes briefly certain technic aspects of the adsorption method for estimating specific surface and also such methods as the bulk density and tinting (of solutions) methods for carbon blacks. Brief mention is also made of a simple light diffraction procedure and certain microscopic technics.

To this reviewer the booklet presents a rather interesting commentary on certain selected particle size technics, but it could well be more comprehensive, even though it may attain the goal indicated by the author in the preface. The literature beyond about 1940 is not covered adequately, particularly with respect to publications in the United States, where many developments in the field have occurred. However, the comments on contributions of foreign authors are of interest to the worker in micromeritics, and make the volume a worthwhile contribution.

H. E. Schweyer
University of Florida
Gainesville, Florida

Methods of Plant Breeding, by Herbert Kendall Hayes, Forrest Rhinehart Immer and David Clyde Smith. 551 pages, ill. McGraw-Hill Book Co., New York, 1955. Price, $8.50.

The authors have made an excellent revision of the original book by H. K. Hayes and F. R. Immer, published in 1942, bringing up to date the important advances in plant breeding made since first publication. Not only have previous chapters been revised, but new material is included of special value to the fundamental aspects of crop breeding, including heterosis, heritability, and centers of origin of cultivated plants. The chapter on breeding forage crops as well as chapters on breeding cotton and sorghum make this book more widely applicable for teaching and reference than the former edition. Some reduction in the chapters on statistics to provide more pages on other phases has not reduced the value of the book, since a full treatment of statistics is available in other standard texts.

Major emphasis in this book, as in the former edition, is on basic principles of plant breeding. To the student of plant breeding this approach is of much greater value than an enumeration of historical accomplishments in this field.

I. J. Johnson
Professor-in-Charge of Farm Crops
Iowa State College
Ames, Iowa

Corn and Corn Improvement, edited by G. F. Sprague, xiv + 699 pp. Academic Press, Inc., New York, 1955. Price $11.50.

An attempt was made to divide the subject matter covered in this monograph into three broad divisions: Breeding, Production, and Utilization. The editor states

that "The particular agronomic audience to be served is the research worker and advanced student interested in corn."

The book contains the following sections on breeding: "Early history of corn and theories as to its origin" by P. Weatherwax, University of Indiana; "Cytogenetic aspects of the origin and evolutionary history of corn" by L. F. Randolph, Cornell University; "Vegetative morphology" by J. E. Sass, Iowa State College; "Structure and development of reproductive organs" by P. Weatherwax; "Cytogenetics of maize" by M. M. Rhoades, University of Illinois; and "Corn breeding" by G. F. Sprague, Iowa State College.

Production phases of corn include: "Mineral nutrition of corn" by J. D. Sayre, Ohio Agricultural Experiment Station; "Climatic requirement" by R. H. Shaw, Iowa State College; "Corn culture" by G. H. Stringfield, Ohio Agricultural Experiment Station; "Production of hybrid corn seed" by J. M. Airy, Pioneer Hi-Bred Corn Company; "Popcorn" by A. M. Brunson, Purdue Agricultural Experiment Station; "Sweet corn" by G. M. Smith, Purdue Agricultural Experiment Station; "Diseases of corn" by A. J. Ullstrup, Purdue Agricultural Experiment Station; and "The most important corn insects" by F. F. Dicke, Federal Corn Borer Laboratory.

Utilization of the crop is divided into "Industrial utilization" by G. F. Sprague and "The nutritive value of corn" by B. H. Schneider, Washington State College.

The reviewer would agree with the editor that "others serving in an editorial capacity might well have chosen a somewhat different array of topics and made quite different space allocations than were used here." The monograph, however, contains such a vast amount of information that every person interested in corn and its improvement should obtain a copy of this book.

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Cereal Chemistry

EDITORIAL POLICY

Cereal Chemistry publishes scientific papers dealing with raw materials, processes, or products of the cereal industries, or with analytical procedures, technological tests, or fundamental research, related thereto. Papers must be based on original investigations, not previously described elsewhere, which make a definite contribution to existing knowledge.

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SUGGESTIONS TO AUTHORS

General. Authors will find the last volume of *Cereal Chemistry* a useful guide to acceptable arrangements and styling of papers. "On Writing Scientific Papers for Cereal Chemistry" (*Trans. Am. Assoc. Cereal Chem.* 6:1–22. 1948) amplifies the following notes.

Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, and all original drawings or photographs for figures. If possible, one set of photographs of figures should also be submitted. Originals can then be held to prevent damage, and the photographs can be sent to reviewers.

Titles and Footnotes. Titles should be specific, but should be kept short by deleting unnecessary words. The title footnote shows "Manuscript received . . ." and the name and address of the author's institution. Author footnotes, showing position and connections, are desirable although not obligatory.

Abstract. A concise abstract of about 200 words follows title and authors. It should state the principal results and conclusions, and should contain, largely by inference, adequate information on the scope and design of the investigation.

Literature. In general, only recent papers need be listed, and these can often be cited more advantageously throughout the text than in the introduction. Long introductory reviews should be avoided, especially when a recent review in another paper or in a monograph can be cited instead.

References are arranged and numbered in alphabetical order of author's names and show author, title, journal, volume, first and last pages, and year. The list is given at the end of the paper. Reference numbers must invariably be cited in the text, but authors' names and year may be cited also. Abbreviations for the names of journals follow the list given in *Chemical Abstracts* **45:** VII–CCLV (1951).

Tables. Data should be arranged to facilitate the comparisons readers must make. Tables should be kept small by breaking up large ones if this is feasible. Only about eight columns of tabular matter can be printed across the page. Authors should omit all unessential data such as laboratory numbers, columns of data that show no significant variation, and any data not discussed in the text. A text reference can frequently be substituted for columns containing only a few data. The number of significant figures should be minimized. Box and side headings should be kept short by abbreviating freely; unorthodox abbreviations may be explained in footnotes, but unnecessary footnotes should be avoided. Leader tables without a number, main heading, or ruled lines are often useful for small groups of data.

Tables should be typed on separate pages at the end of the manuscript, and their position should be indicated to the printer by typing "(TABLE I)" in the appropriate place between lines of the text. (Figures are treated in the same way.)

Figures. If possible, all line drawings should be made by a competent draftsman. Traditional layouts should be followed: the horizontal axis should be used for the independent variable; curves should be drawn heaviest, axes or frame intermediate, and the grid lines lightest; and experimental points should be shown. Labels are preferable to legends. Authors should avoid identification in cut-lines to be printed below the figure, especially if symbols are used that cannot readily be set in type.

All drawings should be made about two to three times eventual reduced size with India ink on white paper, tracing linen, or *blue*-lined graph paper; with any other color, the unsightly mass of small grid lines is reproduced in the cut. Lettering should be done with a guide using India ink; and letters should be 1/16 to 1/8 inch high after reduction.

All original figures should be submitted with one set of photographic reproductions for reviewers, and each item should be identified by lightly writing number, author, and title on the back. Cut-lines (legends) should be typed on a separate sheet at the end of the manuscript. "Preparation of Illustrations and Tables" (*Trans. Am. Assoc. Cereal Chem.* 3: 69–104. 1945) amplifies these notes.

Text. Clarity and conciseness are the prime essentials of a good scientific style. Proper grouping of related information and thoughts within paragraphs, selection of logical sequences for paragraphs and for sentences within paragraphs, and a skillful use of headings and topic sentences are the greatest aids to clarity. Clear phrasing is simplified by writing short sentences, using direct statements and active verbs, and preferring the concrete to the abstract, the specific to the general, and the definite to the vague. Trite circumlocutions and useless modifiers are the main causes of verbosity; they should be removed by repeated editing of drafts.

Editorial Style. A.A.C.C. publications are edited in accordance with *A Manual of Style*, University of Chicago Press, and *Webster's Dictionary*. A few points which authors often treat wrongly are listed below:

Use names, not formulas, for text references to chemical compounds. Use plural verbs with quantities (6.9 g. were). Figures are used before unit abbreviations (3 ml.), and % rather than "per cent" is used following figures. All units are abbreviated and followed by periods, except units of time, which are spelled out. Repeat the degree sign (5°–10°C.). Place 0 before the decimal point for correlation coefficients ($r = 0.95$). Use * to mark statistics that exceed the 5% level and ** for those that exceed the 1% level; footnotes explaining this convention are no longer required. Type fractions on one line if possible, e.g., $A/(B + C)$. Use lower case for farinograph, mixogram, etc., unless used with a proper name, i.e., Brabender Farinograph. When in doubt about a point that occurs frequently, consult the Style Manual or the Dictionary.

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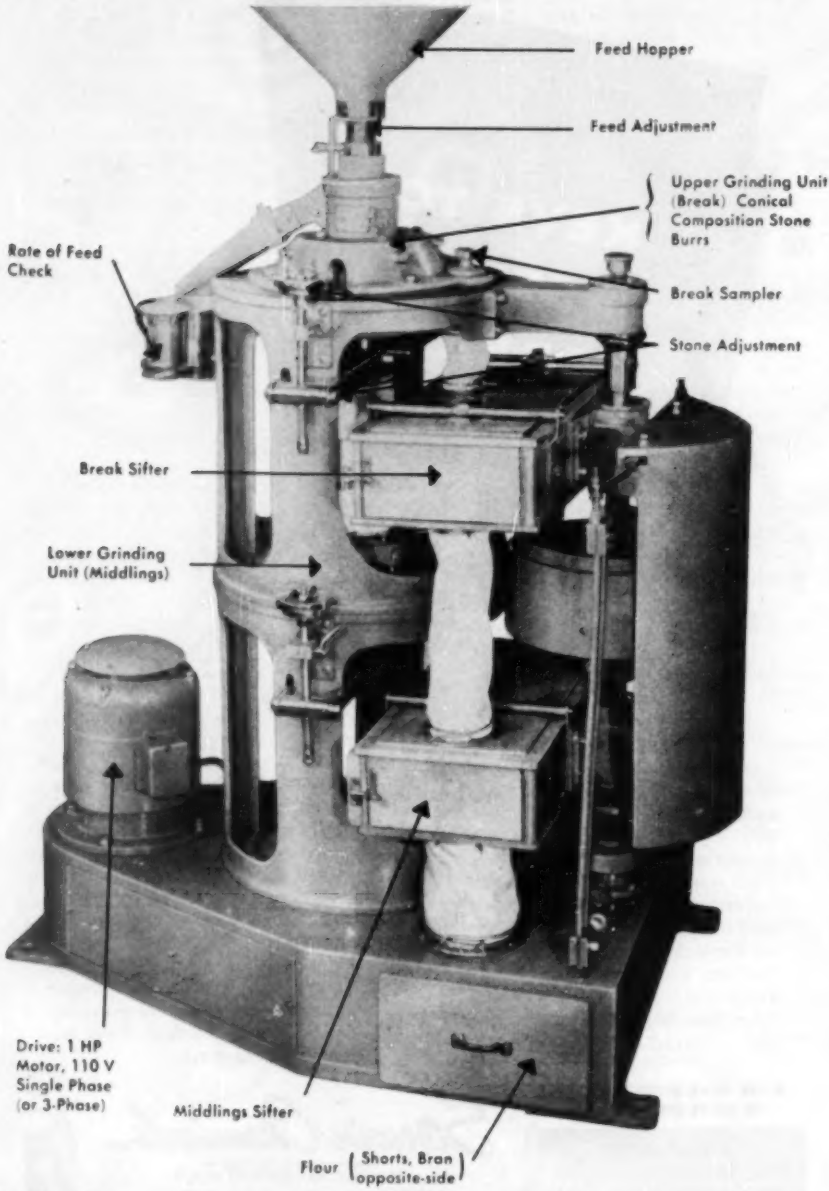

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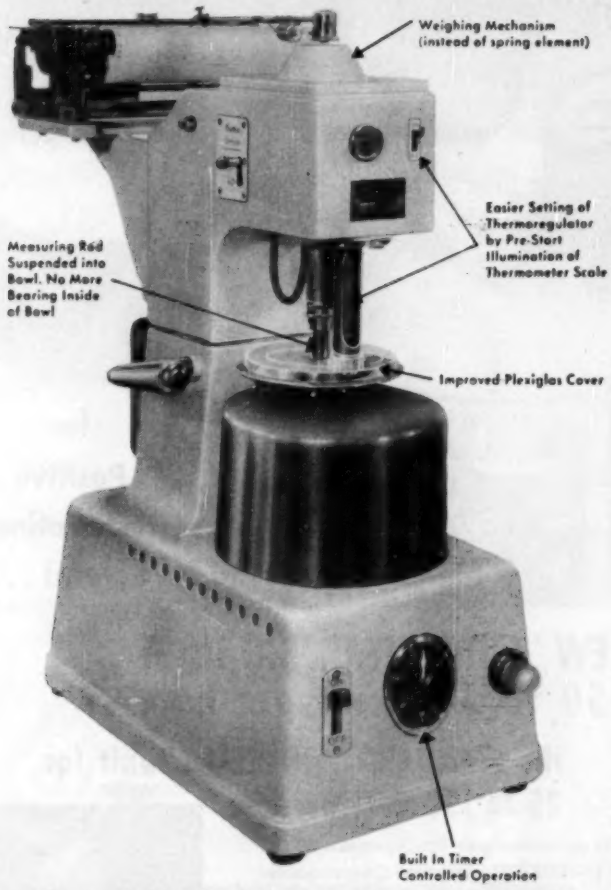

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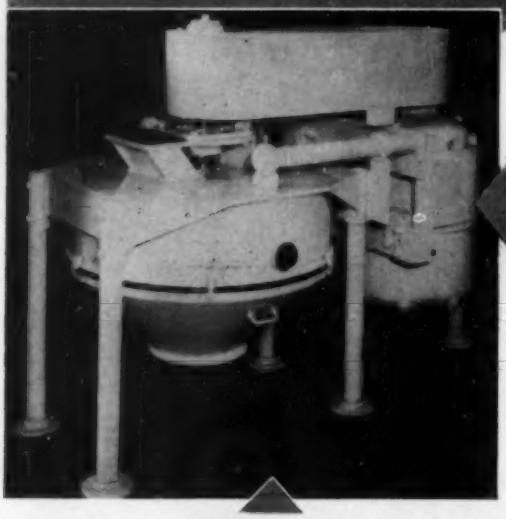

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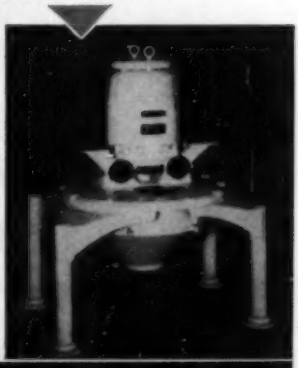

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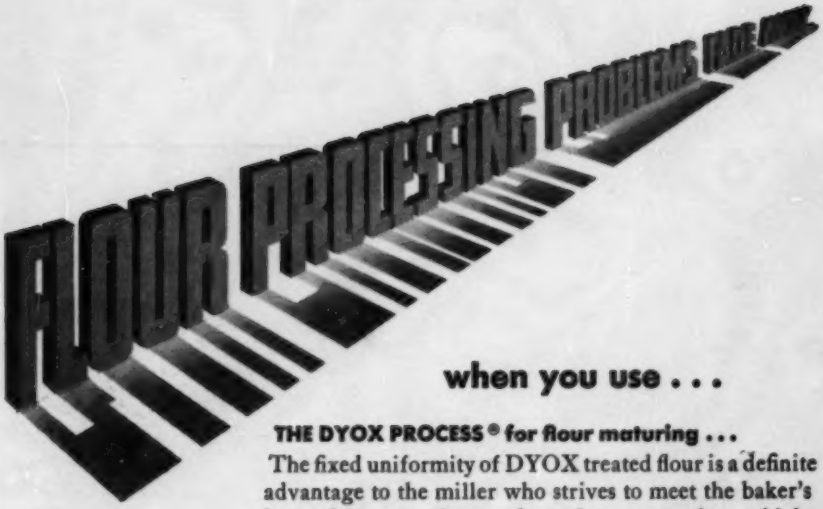

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